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(71) Applicant *(for all designated States except US)*: **BETH ISRAEL DEACONESS MEDICAL CENTER, INC.** [US/US]; 330 Brookline Avenue, Boston, MA 02215 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MOLL, Thomas** [US/US]; 61 Moulton Street, Cambridge, MA 02138 (US). **STROM, Terry, B.** [US/US]; 22 Kennard Road, Brookline, MA 02146 (US). **ZHENG, Xin, Xiao** [CN/US]; 59 Alton Place, Unit 6, Brookline, MA 02446 (US).

(74) Agent: **CREWS, Lee**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

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(54) Title: ANTAGONISTS OF IL-21 AND MODULATION OF IL-21-MEDIATED T CELL RESPONSES

(57) Abstract: The present invention is based, in part, on the creation of molecules that antagonize the receptor for interleukin-21 (IL-21). The antagonist can be, for example, a mutant of IL-21 (mIL-21). Moreover, the mIL-21 polypeptide can be joined to (*e.g.*, joined by way of a peptide bond to) a heterologous polypeptide (*i.e.*, a non-IL-21 polypeptide), such as an Fc region of an immunoglobulin molecule ("mIL-21/Fc"). Such antagonists have been shown to inhibit cellular proliferation in response to either anti-CD3 monoclonal antibodies or anti-CD3 antibodies applied together with IL-2, IL-15, or either cytokine (IL-2 or IL-15) together with IL-21 (these studies and their significance are described further below). Accordingly, the present invention features polypeptides that include a mutant IL-21 sequence, nucleic acids encoding those mutants, compositions containing them, and methods of using them in a variety of diagnostic, prognostic, and treatment regimes.

ANTAGONISTS OF IL-21 AND MODULATION OF IL-21-MEDIATED T CELL RESPONSES

TECHNICAL FIELD

This invention relates to immunology, transplant rejection, and diseases associated with the immune system.

BACKGROUND

Cells within multicellular organisms proliferate and differentiate under the control of various signals, including those carried by hormones and polypeptide growth factors. These molecules are diffusable, and they influence cellular metabolism and other activities by binding to receptors, which are frequently membrane-bound and linked to second messenger systems within the cell.

Cytokines are a particular class of growth factors that stimulate cells within the hematopoietic lineage, and they participate in the immune response and in inflammatory responses. One of the best-known cytokines, erythropoietin (EPO), affects hematopoiesis; thrombopoietin (TPO) stimulates cells within the megakaryocyte lineage; and granulocyte-colony stimulating factor (G-CSF) stimulates neutrophils. Thus, these cytokines help restore blood cell levels to normal in patients who are anemic, who are suffering from thrombocytopenia or neutropenia, or who are receiving chemotherapy.

There are many other cytokines, and new members of this class of molecules continue to be discovered. For example, interleukin-21 (IL-21) was recently discovered by way of its receptor (*see,* Parris-Novak *et al.*, *Nature* 408:57-63, 2000; *see also* Ozaki *et al.*, *Proc. Natl. Acad. Sci. USA* 97:11439-11444, 2000; Voβhenrich and Di Santo, *Curr. Biol.* 11:R175-R177, 2001; and Asao *et al.*, *J. Immunol.* 167:1-5, 2001). IL-21 is thought to stimulate the proliferation of natural killer (NK) cells, mature B cells, and T cells (Parrish-Novak *et al.*, *Nature* 408:57-63, 2000). Human IL-21 consists of 162 amino acids, with a predicted signal sequence terminating at Gly31. The mature IL-21 protein therefore consists of 131 residues that form a four-helix-bundle and exhibit homology with IL-2, IL-4, and IL-15. Both the human and murine IL-21 proteins have an acidic amino acid in position Asp13 of the mature peptide, which corresponds to Glu9 in IL-4, which is involved in the primary high-

affinity interaction between IL-4 and IL-4Rα. Human and murine IL-21 proteins also contain a conserved Gln114, and it has been reported that this amino acid residue is equivalent to Gln141 of IL-2, which has been implicated in the interaction between IL-2 and IL-2Rγc (Parrish-Novak *et al.*, *Nature* 408:57-63, 2000; Zurawski *et al.*, *EMBO J.* 12:5113-5119, 1993).

SUMMARY

The present invention is based, in part, on the creation of molecules that antagonize the receptor for interleukin-21 (IL-21). The antagonist can be, for example, a mutant of IL-21 (mIL-21). Moreover, the mIL-21 polypeptide can be joined to (*e.g.*, joined by way of a peptide bond to) a heterologous polypeptide (*i.e.*, a non-IL-21 polypeptide), such as an Fc region of an immunoglobulin molecule ("mIL-21/Fc"). Such antagonists have been shown to inhibit cellular proliferation in response to either anti-CD3 monoclonal antibodies or anti-CD3 antibodies applied together with IL-2, IL-15, or either cytokine (IL-2 or IL-15) together with IL-21 (these studies and their significance are described further below). Accordingly, the present invention features polypeptides that include a mutant IL-21 sequence, nucleic acids encoding those mutants, compositions containing them, and methods of using them in a variety of diagnostic, prognostic, and treatment regimens.

The invention also features compositions containing, as a first agent, an IL-21 antagonist and, as a second agent, a polypeptide that specifically binds a receptor for an interleukin other than IL-21. The first agent can be, for example, a polypeptide comprising a mutant IL-21 sequence, an antibody that specifically binds and thereby inhibits an activity of an IL-21 receptor, or a soluble IL-21 receptor. The second agent can be, for example, a polypeptide comprising a mutant IL-15, an antibody that specifically binds (and thereby inhibits an activity of) IL-15 or an IL-15 receptor, an antibody that specifically binds (and thereby inhibits an activity of) IL-2 or an IL-2 receptor, or an IL-2 receptor agonist (*e.g.*, IL-2 (or a biologically active fragment or mutant thereof), IL-2/Fc, or an antibody that specifically binds an IL-2 receptor and thereby functions as an IL-2 agonist). Where the first agent is a polypeptide comprising a mutant IL-21/Fc fusion protein and the second agent is a heterologous fusion protein (*e.g.*, a mutant IL-15/Fc fusion protein), these two agents can combine to form a single

dimeric molecule (*e.g.*, a dimer that includes a mutant IL-21 fused at its C terminus to an Fc portion of an immunoglobulin and a mutant IL-15 fused at its C terminus to an Fc portion of an immunoglobulin; dimerization being mediated by interaction between amino acid residues in the respective Fc regions).

Without limiting the invention to agents that work by any particular mechanism, we note that agents that include an Fc portion can promote antibody dependent cell-mediated cytotoxicity (ADCC) or complement directed cytotoxicity (CDC) and that IL-15 antagonists can promote passive cell death. Additional combinations of agents are described below (*e.g.*, the compositions of the invention can include (in addition to, or in place of, an IL-15 antagonist), any other factor required for T cell survival (*e.g.*, IL-4, IL-7, OX-4 ligand, IFN-β, 4-1BB, or IGF-I)).

We also note here that, generally, the various compositions of the invention will include an IL-2 receptor agonist (*e.g.*, IL-2 (or a biologically active fragment or mutant thereof), IL-2/Fc, or an antibody that binds to and functions as an agonist of an IL-2 receptor) when one intends to suppress an immune response or to induce tolerance by (at least in part) provoking activation induced cell death (AICD). However, as IL-2 (or an IL-2 receptor agonist) also stimulates T cell proliferation, and as this is undesirable when attempting to suppress an immune response, compositions that include IL-2 (or an IL-2 receptor agonist) may also include an agent that inhibits T cell proliferation (*e.g.*, rapamycin or mycophenolate mofetil (MMF), azathioprine, or any of the other agents known and used in the art to prevent cellular proliferation (including chemotherapeutic agents)) (Rabinovitch *et al.*, *Diabetes* 51:638-645, 2002).

Another agent that promotes AICD is the Fas Ligand (FasL).

In alternative embodiments, the compositions of the invention can include (in place of, or in addition to, one or more of the agents described above), agents that inhibit the expression of the nucleic acids (*e.g.*, DNA or RNA) that encode an interleukin (*e.g.*, IL-21, IL-2, or IL-15) or an interleukin receptor (*e.g.*, an IL-21, IL-2, or IL-15 receptor). Similarly, where one wishes to elicit AICD, one can employ or administer a nucleic acid that encodes a protein that does so (*e.g.*, IL-2).

When two or more agents are employed (circumstances in which the compositions of the invention are useful are described further below), they may or

may not be physically separate from one another. An agent can be a single entity that has primarily one functional activity (*e.g.*, an antibody that targets IL-21, IL-2 or IL-15 by specifically binding IL-21, IL-2, or IL-15 or their respective receptors), but it can also be a single entity that has at least two functional activities (*e.g.*, IL-21/Fc, IL-2/Fc, mIL-15/Fc, or an anti-IL-21R, anti-IL-2R or anti-IL-15R antibody). In these molecules, the interleukin portion acts as a cytokine antagonist or agonist and the Fc portion of the molecule can mediate CDC and ADCC. Thus, a composition that includes (1) an agent that induces AICD, (2) an agent that induces CDC, and (3) an agent that inhibits cellular proliferation may include only two active ingredients (*e.g.*, (1) an IL-2/Fc molecule, which induces AICD and CDC, and (2) rapamycin, which inhibits cellular proliferation).

The compositions described herein are useful in treating patients who would benefit from immune suppression. For example, they are useful in treating a patient who has received, or is scheduled to receive, a transplant or a patient who is suffering from graft versus host disease (GVHD)). GVHD is characterized by a response of donor leukocytes against antigens in the recipient. This response is particularly problematic in bone marrow transplants, but also occurs in whole organ transplants; donor leukocytes resident in transplanted organs are always co-transplanted. The compositions can also be used to treat a patient who has an immune disease, particularly an autoimmune disease; a patient who has cancer (*e.g.*, a cancer of the immune system); a patient who has suffered a vascular injury (whether caused by an inflammatory disease or process, trauma or a surgical procedure such as a reconstructive surgery or balloon angioplasty); or a patient who has been infected with a human immunodeficiency virus (HIV).

Although the compositions of the invention can contain more than one agent, the methods of the invention are not limited to those in which the agents are administered simultaneously. A patient or a cell or organ in culture can be treated with one agent and then another. For example, a patient can receive a composition containing an IL-21 antagonist before receiving a composition containing IL-2, an IL-2 antagonist, or an IL-15 antagonist (*e.g.* a mutant IL-15 or a mutant IL-15/Fc). Similarly, a patient can receive a composition containing rapamycin before receiving a composition containing an IL-2 agonist. Moreover, the compositions of the

invention (applied simultaneously or sequentially) can be used to treat an organ or cellular graft before it is implanted in a patient. The agents of the invention, and methods for their use, are described further below.

Many of the agents used in the context of the present invention have advantageous therapeutic characteristics. For example, agents that target an IL-21 receptor or an IL-15 receptor can be proteins (*e.g.*, fusion proteins) that include a mutant IL-21 or a mutant IL-15 polypeptide (*e.g.*, mIL-21/Fc or mIL-15/Fc). These agents are unlikely to be immunogenic because the mutant portion of the fusion protein can differ from the corresponding wild type protein by only a few substituted residues. Despite the change from a wild type sequence, these mutants can compete effectively with IL-21 or IL-15 for their respective receptors. Further, agents of the invention can activate components of the host immune system, such as complement and phagocytes, which ultimately mediate elimination of (or depletion of) cells bearing the receptor (*e.g.*, an IL-21, IL-2, or IL-15 receptor) to which the agent binds. For example, agents of the invention can mediate lysis or phagocytosis of targeted cells. As the alpha subunit of the IL-15 receptor (IL-15Rα) is expressed by activated or malignant immune cells, but not by resting immune cells, agents of the invention can be used to specifically target those cells that have been activated (*e.g.*, antigen-activated T cells) or that have become malignant. Thus, although T cells represent a preferred target for the agents of the invention, the compositions of the invention can also be used to target other cells involved in the pathogenesis of immunological disorders, such as other cells of the immune system or hyperproliferating cells of tissues.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. Other features and advantages of the invention will be apparent from the drawings, the detailed description, and claims. Although materials and methods similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred materials and methods are described below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and 1B represent various interleukin alignments. In Fig. 1A, human and murine IL-21 (hIL-21 (SEQ ID NO:1) and muIL-21 (SEQ ID NO:2), respectively) are aligned with human IL-15, IL-4 and IL-2 (hIL-15 (SEQ ID NO:3), hIL-4 (SEQ ID NO:4), and hIL-2 (SEQ ID NO:5), respectively). Fig. 1A is reproduced from Parrish-Novak *et al.* (*Nature* 408:57-63, 2000). Fig. 1B shows an alternative alignment highlighting Q119, the amino acid residue that is important for gamma chain interaction (hIL-21 (SEQ ID NO:6); muIL-21 (SEQ ID NO:7); hIL-15 (SEQ ID NO:8); hIL-2 (SEQ ID NO:9).

Fig. 2 is a representation of a wild type murine IL-21/Fc nucleic acid sequence (SEQ ID NO:10) and the predicted amino acid sequence (SEQ ID NO:11).

Fig. 3 is a representation of a mutant murine IL-21/Fc nucleic acid sequence (SEQ ID NO:12) and the predicted amino acid sequence (SEQ ID NO:13).

Fig. 4 is a representation of a wild type IL-15 nucleic acid sequence (SEQ ID NO:14) and the predicted amino acid sequence (SEQ ID NO:15).

Fig. 5 is a representation of a mutant IL-15 nucleic acid sequence (SEQ ID NO:16) and the predicted amino acid sequence (SEQ ID NO:17). The wild-type codon encoding glutamine at position 149, CAG, and the wild-type codon encoding glutamine at position 156, CAA, have both been changed to GAC, which encodes aspartate. (These positions (149 and 156) correspond to positions 101 and 108, respectively, in the mature IL-15 polypeptide, which lacks a 48-amino acid signal sequence).

Figs. 6A and 6B are illustrations of IL-21/Fc fusion proteins. The IL-21 portion may contain mutations, as described herein and as illustrated in Fig. 6B.

Fig. 7 is a line graph depicting the effect of IL-21/Fc and mutant IL-21/Fc on peripheral blood mononuclear cell (PBMC) proliferation in culture. The mutant IL-21/Fc chimera blocks anti-CD3 triggered PBMC proliferation, but the wild type IL-21/Fc chimera does not.

Fig. 8 is a line graph depicting the effect of various interleukin-containing compositions on PBMC proliferation in culture. The compositions tested included recombinant IL-2 (rIL-2); recombinant IL-2 plus IL-21/Fc (rIL-2 + IL-21/Fc); recombinant IL-2 plus mutant IL-21/Fc (rIL2 + mutant IL-21/Fc); and recombinant

IL-2 plus IL-21/Fc plus mutant IL-21/Fc (rIL-2 + IL-21/Fc + mutant IL-21/Fc). Mutant IL-21/Fc blocked anti-CD3-triggered, IL-2-triggered, and IL-21-triggered PBMC proliferation in culture.

Fig. 9 is a line graph depicting the effect of various interleukin-containing compositions on PBMC proliferation in culture. The compositions tested included recombinant IL-15 (rIL-15); recombinant IL-15 plus IL-21/Fc (rIL-15 + IL-21/Fc); recombinant IL-15 plus mutant IL-21/Fc (rIL-15 + mutant IL-21/Fc); and recombinant IL-15 plus IL-21/Fc plus mutant IL-21/Fc (rIL-15 + IL-21/Fc + mutant IL-21/Fc). Mutant IL-21/Fc blocked anti-CD3-triggered, IL-15-triggered, and IL-21-triggered PBMC proliferation in culture.

DETAILED DESCRIPTION

The present invention features compositions and methods for inhibiting (or suppressing) an immune response (*e.g.*, a humoral or a cellular immune response). The compositions include an IL-21 antagonist (*e.g.* a mutant IL-21 polypeptide), which can be administered to treat patients in a number of circumstances. For example, a composition containing an IL-21 antagonist can be administered to a patient who has received (or is scheduled to receive) a transplant of a biological organ, tissue, or cell; to a patient who has graft versus host disease; or to a patient who has an immune disease (*e.g.*, an autoimmune disease). The IL-21 antagonist may, but need not, fully block one or more of the cellular events known to occur when wild type IL-21 binds an IL-21R-bearing cell. The antagonist is useful and within the scope of the present invention so long as it confers a benefit to a patient to whom it is administered, either directly or indirectly (as would occur when a patient receives a cell, tissue, or organ that has been treated with an interleukin antagonist). The benefit to the patient can be an objective or subjective improvement in a clinical sign or symptom. The clinical sign can be any indication that the antagonist has inhibited (or suppressed) an immune response (*e.g.*, a reduction in the number of antigen-reactive T cells), and the clinical symptom can be any indication that the antagonist has improved the patient's health (*e.g.*, an agent is an IL-21 antagonist if, when administered to a patient, the frequency or severity of a symptom associated with an immune disease (or any of the conditions described herein) is lessened).

IL-21 antagonists can inhibit an immune response by inhibiting either the expression or activity of IL-21 or the expression or activity of its receptor. An IL-21 antagonist can be used alone or more than one type of IL-21 antagonist can be used together. For example, one can administer an agent that inhibits the expression of IL-21 (*e.g.*, an antisense molecule or an siRNA) and a mutant IL-21 polypeptide that binds to, and thereby inhibits the activity of, an IL-21 receptor. Alternatively, one or more IL-21 antagonists can be used in combination with at least one other agent, such as an agent that targets IL-15 or an IL-15R or an agent that targets IL-2 or an IL-2R (modes of administration, including *ex vivo* treatment of grafts, are known in the art and described further below).

More generally, one can inhibit an immune response by activating signaling pathways that lead to the death of activated T cells (by, *e.g.*, AICD); depriving cells of factors that are required for their survival (cells that die following such deprivation are said to die by passive cell death); or targeting activated cells for lysis by components of the immune system (cells that die in this way are said to die by ADCC or CDC). Accordingly, the compositions of the invention include agents that achieve one or more of these ends (*i.e.*, that promote T cell death via a recognized cell death pathway (*e.g.*, AICD, passive cell death, ADCC, or CDC)). In addition to containing one or more agents that promote T cell death, the compositions of the invention can include one or more agents that inhibit T cell proliferation (as occurs, *e.g.*, in response to an antigen). For example, the invention features a composition (*e.g.*, a pharmaceutically acceptable composition or one formulated for application to an organ or cell culture) that includes an IL-21 antagonist, IL-2/Fc (which, for example, promotes AICD and cellular lysis via ADCC or CDC), and/or mIL-15/Fc (which antagonizes IL-15 (and thereby promotes passive cell death) and promotes cellular lysis via ACDD or CDC), and rapamycin (which inhibits T cell proliferation).

The term "agent" is meant to encompass essentially any type of molecule (whether complete (*e.g.*, a full-length polypeptide) or a portion thereof) that can be used as a therapeutic agent. Proteins, such as antibodies, fusion proteins, homodimers, heterodimers, and soluble ligands or receptors, any of which may either be identical to a wild type protein or contain a mutation (*i.e.*, a deletion, addition, or substitution of one or more amino acid residues), and the nucleic acid molecules that

encode them (or that are "antisense" to them; *e.g.*, an oligonucleotide that is antisense to the nucleic acids that encode IL-21, IL-2, IL-15, or a component (*e.g.*, a subunit) of their receptors), are all "agents." The agents of the invention can either be administered systemically, locally, or by way of cell-based therapies (*i.e.*, an agent of the invention can be administered to a patient by administering a cell that expresses that agent to the patient). The cell can be a cell administered to the patient solely for the purpose of expressing the therapeutic agent. The cell can also be a cell of a cellular, tissue, or organ transplant. For example, transplanted cells (*e.g.*, islet cells) or cells within tissues or organs (*e.g.*, cells within a patch of skin or a liver, kidney, or heart) can be treated with an agent or transduced with a nucleic acid molecule that encodes an agent *ex vivo* (*e.g.*, prior to transplantation). In this way, the transplanted cell produces its own immunosuppressive agents. For example, a cell with a desirable phenotype (*e.g.*, an insulin producing cell) can be modified to include a gene producing one or more of the immunosuppressive factors or agents of the invention. The transplanted cell, tissue, or organ can be treated either prior to or subsequent to transplantation. Methods of administering agents to patients (or to cells or organs in culture) are known and routinely used by those of ordinary skill in the art and are discussed further below.

Agents that Target IL-21 (or an IL-21R) or IL-15 (or an IL-15R)

The compositions of the invention can include one or more agents that target IL-21 (or an IL-21 receptor) or IL-15 (or an IL-15 receptor). As noted above, a single agent (*e.g.*, an IL-21 antagonist) can have multiple functional domains. Agents that target IL-21 or an IL-21R include agents that bind to (or otherwise interact with) IL-21, an IL-21R, or the nucleic acids that encode them, as well as agents that bind to and subsequently destroy IL-21R-bearing cells, such as activated T cells. Thus, agents useful in achieving immune suppression can contain two functional moieties: a targeting moiety that targets the agent to an IL-21R-bearing cell and a target-cell depleting (*e.g.*, lytic) moiety that leads to the elimination of the IL-21R-bearing cell. In one embodiment, the targeting moiety binds an IL-21R without effectively transducing a signal through that receptor. For example, the targeting moiety can include a mutant IL-21 polypeptide. "Targeting" occurs when an agent directly or

indirectly binds to, or otherwise interacts with, an interleukin or an interleukin receptor in a way that affects the activity of the interleukin or the interleukin receptor. Activity can be assessed in *in vivo* or *ex vivo* assays by one of ordinary skill in the art. For example, one can assess the strength of signal transduction or another downstream biological event that occurs, or would normally occur, following receptor binding (in, for example, the leukemic cell lines MOLT-14, YT, or HuT-102). The activity generated by an agent that targets an interleukin or an interleukin receptor can be, but is not necessarily, different from the activity generated when a naturally occurring interleukin binds a naturally occurring interleukin receptor. For example, an agent that targets an IL-2 receptor falls within the scope of the invention even if that agent generates substantially the same activity that would occur had the receptor been bound by naturally occurring IL-2. When an agent generates activity that is substantially the same as, or greater than, the activity generated by a naturally occurring ligand, the agent can be described as a receptor agonist (the agent and the natural ligand being examined under the same conditions). When an agent generates activity that is less than the activity generated by a naturally occurring ligand, the agent can be described as an antagonist of the receptor (if the agent's primary interaction is with the receptor; *e.g.*, mIL-21 or mIL-15) or of the interleukin (if the agent's primary interaction is with the interleukin; *e.g.*, an anti-IL-21 antibody or an anti-IL-15 antibody). Here again, levels of activity are assessed by testing the agent and the naturally occurring receptor (or ligand) under the same conditions.

In addition to a targeting moiety, an agent of the invention can include a target-cell depleting moiety that lyses or otherwise eliminates the target cell. The depleting moiety can be, for example, the Fc region of an immunoglobulin molecule. The Fc region can be derived from an IgG, such as human IgG1, IgG2, IgG3, IgG4, or analogous mammalian IgGs or from an IgM, such as human IgM or analogous mammalian IgMs. In a preferred embodiment, the Fc region includes the hinge, CH2 and CH3 domains of human IgG1 or murine IgG2a. The comments made here regarding the Fc region are not limited to IL-21 antagonists; they are equally applicable to other interleukins and interleukin-derived molecules useful in the present invention (*e.g.*, to IL-15 (*e.g.*, mIL-15/Fc molecules) and IL-2 (*e.g.*, IL-2/Fc)). Although the invention is not limited to agents that work by any particular

mechanism, it is believed that the Fc region mediates complement and phagocyte-driven elimination of IL-21R-bearing cells.

Thus, an agent can be a chimeric polypeptide that includes a mutant interleukin polypeptide (*e.g.*, an IL-21 or an IL-15 polypeptide) and a heterologous polypeptide such as the Fc region of an immunoglobulin molecule (*e.g.*, the IgG or IgM subclasses of antibodies). The Fc region may include a mutation that inhibits complement fixation and Fc receptor binding, or it may be target-cell depleting (*i.e.*, able to destroy cells by binding complement or by another mechanism, such as antibody-dependent complement lysis).

The Fc region can be isolated from a naturally occurring source, recombinantly produced, or synthesized (as can any polypeptide featured in the present invention). For example, an Fc region that is homologous to the IgG C-terminal domain can be produced by digestion of IgG with papain. IgG Fc has a molecular weight of approximately 50 kDa. The polypeptides of the invention can include the entire Fc region, or a smaller portion that retains the ability to lyse cells. In addition, full-length or fragmented Fc regions can be variants of the wild-type molecule. That is, they can contain mutations that may or may not affect the function of the polypeptide.

While the Fc region can be "target-cell depleting," it may also be "non-target-cell depleting" (*e.g.*, it may be non-lytic). A non-target-cell depleting Fc region typically lacks a high affinity Fc receptor binding site and a C'1q binding site. The high affinity Fc receptor binding site of murine IgG Fc includes the Leu residue at position 235 of IgG Fc. Thus, the murine Fc receptor binding site can be destroyed by mutating or deleting Leu 235. For example, substitution of Glu for Leu 235 inhibits the ability of the Fc region to bind the high affinity Fc receptor. The murine C'1q binding site can be functionally destroyed by mutating or deleting the Glu 318, Lys 320, and Lys 322 residues of IgG. For example, substitution of Ala residues for Glu 318, Lys 320, and Lys 322 renders IgG1 Fc unable to direct antibody-dependent complement lysis. In contrast, a target-cell depleting IgG Fc region has a high affinity Fc receptor binding site and a C'1q binding site. The high affinity Fc receptor binding site includes the Leu residue at position 235 of IgG Fc, and the C'1q binding site includes the Glu 318, Lys 320, and Lys 322 residues of IgG1. Target-cell depleting

IgG Fc has wild type residues or conservative amino acid substitutions at these sites. Target-cell depleting IgG Fc can target cells for antibody dependent cellular cytotoxicity or complement directed cytolysis (CDC). Appropriate mutations for human IgG are also known (*see, e.g.*, Morrison *et al.*, *The Immunologist* 2:119-124, 1994; and Brekke *et al.*, *The Immunologist* 2:125, 1994).

The interleukin or interleukin-derived (*e.g.*, mutant) polypeptides described herein (*e.g.*, agents that target the IL-21R or the IL-15R) can be fused to an antigenic tag (*e.g.*, a FLAG sequence). FLAG sequences are recognized by biotinylated, highly specific, anti-FLAG antibodies (*see* U.S. Patent No. 6,001,973; *see also* Blanar *et al.*, *Science* 256:1014, 1992, and LeClair *et al.*, *Proc. Natl. Acad. Sci. USA* 89:8145, 1992).

Mutant IL-21 polypeptides that bind the IL-21 receptor complex with a high affinity, but fail to fully activate signal transduction, have been produced. These mutant polypeptides will compete effectively with wild type IL-21 polypeptides and can inhibit one or more of the events that normally occur in response to IL-21 signaling, such as cellular proliferation. Here again, analogous statements can be made with respect to IL-15. Mutant IL-15 polypeptides have been produced, as described in U.S. Patent No. 6,001.973 (which is incorporated by reference herein in its entirety). The "wild type" polypeptides referred to herein are polypeptides that are identical to a naturally occurring interleukin (*e.g.*, a wild type IL-21, IL-15, or IL-2). In contrast, a "mutant" polypeptide is a polypeptide that has at least one mutation relative to the corresponding wild type interleukin (*e.g.*, a mutant IL-21 polypeptide has at least one mutation with respect to wild type IL-21) and that inhibits at least one of the *in vivo* or *in vitro* activities that are usually promoted by the wild type polypeptide.

While a mutant polypeptide that can be used according to the present invention will generally block at least 40% (*e.g.*, 50%, 60%, 70%, 80% 90%, 95%, 98%, or 100%) of one or more of the activities of the corresponding wild type molecule (the mutant polypeptide and the wild type polypeptide being tested under the same conditions), mutants that block activity to a lesser extent may still be within the scope of the invention. As noted above, a mutant polypeptide falls within the scope of the invention so long as it confers a benefit to a patient to whom it is

administered. The ability of a mutant polypeptide (*e.g.*, a mutant IL-21 or mutant IL-15 polypeptide) to block wild type activity can be assessed in numerous assays, including those known in the art and those described herein. For example, resting B lymphocytes (also known simply as B cells) express IL-21R and can be used to assess IL-21 antagonists. For example, although IL-21 alone has no mitogenic effect on isolated human B cells, it significantly co-stimulates proliferation induced by anti-CD40 monoclonal antibodies (Parrish-Novak *et al.*, *Nature* 408:57-63, 2000). Accordingly, one can assess B cell proliferation following stimulation with anti-CD40 monoclonal antibodies and compositions containing either wild type IL-21 or a mutant IL-21. If proliferation is inhibited by the mutant IL-21 (relative to the proliferation observed with wild type IL-21 under the same conditions), the mutant inhibits at least one of the cellular events that normally occurs when wild type IL-21 specifically binds to an IL-21 receptor complex and it is, therefore, within the scope of the present invention. Alternatively, the proliferation of T cells following stimulation with anti-CD3 monoclonal antibodies (either in the presence or absence of IL-2 and/or IL-15), as described herein, can be assessed in the presence of compositions containing either wild type IL-21 or a mutant IL-21. Similarly, mutant IL-15 polypeptides can be tested in the BAF-BO3 cell proliferation assay described herein (in which the cells are transfected with a construct encoding IL-2Rβ) and in *in vivo* models of transplantation and autoimmune disease.

A mutation in a polypeptide (*e.g.*, an interleukin or its receptor) can consist of a change in the number or content of amino acid residues. For example, a mutant IL-21 or a mutant IL-15 can have a greater or a lesser number of amino acid residues than wild type IL-21 or wild type IL-15, respectively. Alternatively, or in addition, the mutant polypeptide can contain a substitution of one or more amino acid residues that are present in the corresponding wild type protein. The substitution may be "one-for-one" (a single amino acid residue may take the place of a single amino acid residue in the wild type protein) or more than one "new" amino acid residue may be substituted in place of a single amino acid in the wild type protein. For example, a mutant IL-15 polypeptide can differ from wild type IL-15 by the addition, deletion, or substitution of a single amino acid residue at position 156, position 149, or both. For example, the mutant IL-15 polypeptide can differ from wild-type IL-15 by the

substitution of aspartate for glutamine at residues 156 and 149 (compare Fig. 4 and Fig. 5).

Mutant polypeptides useful as targeting agents, like wild type IL-15, recognize and bind a component of the IL-15R. In one embodiment, the mutation of IL-15 is in the carboxy-terminal domain of the cytokine, which is believed to bind IL-2Rγ (the IL-2 receptor γ subunit). Alternatively, or in addition, mutant IL-15 polypeptides can include one or more mutations within IL-2Rβ (the IL-2 receptor ß subunit) binding domain. Similarly, a mutant IL-21 polypeptide can differ from wild type IL-21 by an addition, deletion, or substitution of a single amino acid residue at position 119, position 114, or both. For example, one can replace the glutamine residue at position 119, the glutamine residue at position 114, or both, with an asparate residue (or aspartate residues).

In the event a mutant interleukin polypeptide contains a substitution of one amino acid residue for another, the substitution can be, but is not necessarily, a conservative substitution, which includes a substitution within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Instead of using, or in addition to using, an interleukin as a targeting polypeptide (*e.g.*, a mutant IL-21 or mutant IL-15 polypeptide), the therapeutic agent (or interleukin antagonist) can be an antibody. For example, either IL-21 or IL-15 can be targeted (*i.e.*, specifically bound) with an antibody. Similarly, the receptors for these cytokines can be targeted with antibodies that bind a component of the receptor (*e.g.*, the IL-15Rα subunit). The methods by which antibodies, including humanized antibodies, can be generated against a component of a receptor are well known in the art. The antibodies preferably should be able to activate complement and phagocytosis, for example, human IgG3 and IgG1 (preferably the latter) subclasses, or murine IgG2a subclass.

The methods of the invention can also be carried out with compositions that contain: (a) two or more agents, each of which promote T cell death or (b) at least one agent that promotes T cell death and at least one agent that inhibits T cell proliferation. The agent that promotes T cell death can do so by promoting passive cell death, which occurs when a T cell is deprived of a factor required for its survival.

IL-15 is one such agent (others are described below). Thus, agents that interfere with the ability of IL-15 to serve as a survival factor (*e.g.*, an antibody that specifically binds to IL-15 or the IL-15 receptor) can be included in the compositions of the invention (*e.g.*, a composition can include an agent that promotes AICD, an agent that promotes passive cell death (*e.g.*, an anti-IL-15 antibody), and, optionally, an agent that inhibits T cell proliferation.

In addition, soluble interleukin receptors (*e.g.*, an IL-15R α chain or a soluble IL-21 receptor (*e.g.*, all or a portion of the extracellular domain(s) of the IL-21R sufficient to bind IL-21) can be used as antagonists. As the name implies, soluble receptors are not bound to a cell membrane; typically, they lack transmembrane and cytoplasmic domains. Soluble receptors can include non-receptor sequence, such as affinity tags to facilitate purification, sequences that can be used to attach the polypeptide to a substrate, or immunoglobulin constant region sequences. While the IL-15 receptor complex consists of $\alpha\beta\gamma$ subunits, the α chain alone displays a high affinity for IL-15. Thus, soluble IL-15R α chain will bind IL-15 and prevent IL-15 from binding to a cell surface-bound IL-15R complex. Thus, a soluble IL-15R α chain can act as a receptor-specific antagonist. Soluble IL-21 receptors can be used in combination with an IL-15 antagonist, and compositions containing such agents (*i.e.*, a soluble IL-21 receptor and an IL-15 antagonist) are within the scope of the present invention. Similarly, the invention features compositions comprising a soluble IL-21 receptor, an IL-2 agonist, an agent that inhibits cellular proliferation, and, optionally, an IL-15 antagonist.

Construction of soluble IL-21R involves cloning the extracellular fragment of the IL-21R from receptor-positive cells, such as activated T cells or receptor expressing cell lines, and, optionally, fusing it to a molecular tag sequence. The tag sequence can be, for example, FLAG, GST, or Histidine. This genetic construct in an expression vector can be transfected into expressing cell lines. The tagged soluble IL-21R produced by expressing cell lines will be purified using mAbs specific for the Tag sequence. Furthermore, an IL-21 extracellular domain can be linked (*e.g.*, fused by way of a peptide bond) to an immunoglobulin Fc domain (*e.g.* hinge, CH2 and CH3 domains of Immunoglogulin G), preferably of an IgG or IgM subtype. Such a fusion protein could be expressed in a suitable cell type, many of which are known to

those of ordinary skill in the art and could be purified using, for example, Protein A Sepharose™ or similar affinity purification procedures.

Construction of soluble IL-15R α chain involves cloning the extracellular fragment of the IL-15R α chain from receptor-positive cells, such as activated T cells or receptor expressing cell lines, and, optionally, fusing it to a molecular tag sequence. The tag sequence can be, for example, FLAG, GST, or Histidine. This genetic construct in an expression vector can be transfected into expressing cell lines. The tagged soluble IL-15R α chain produced by expressing cell lines will be purified using mAbs specific for the Tag sequence. Furthermore, an IL-15 extracellular domain can be linked (*e.g.*, fused by way of a peptide bond) to an immunoglobulin Fc domain (*e.g.* hinge, CH2 and CH3 domains of Immunoglogulin G), preferably of an IgG or IgM subtype. Such a fusion protein could be expressed in a suitable cell type, many of which are known to those of ordinary skill in the art and could be purified using, for example, Protein A Sepharose™ or similar affinity purification procedures.

Agents that Target IL-2 or an IL-2 Receptor

The IL-21 and/or IL-15 antagonists described above can be combined with or administered with agents that target IL-2 or an IL-2R. As noted, an agent that is administered "with" another may be, but is not necessarily, administered at the same time or in the same manner. For example, an antagonist of IL-21 or IL-15 may be administered before or after an agent that targets an IL-2R. Similarly, an agent that targets IL-21, IL-15 or their receptors can be administered *ex vivo* (to treat, for example, a cell, tissue, or organ that is slated for transplantation) while an agent that targets IL-2 or an IL-2R can be administered systemically (*e.g.*, intravenously) to a patient (*e.g.* a patient who has received a transplant that was treated *ex vivo* with an agent that targets IL-21 or IL-15). Similarly, one can administer an agent that promotes AICD at a different time or in a different manner than an agent that inhibits cellular proliferation. Thus, in the methods of the invention, any of the agents or types of molecules that are combined in the compositions of the invention can be administered separately.

To inhibit an IL-2R, one can administer any agent that binds to and antagonizes IL-2 or an IL-2R. Agents that target IL-2 or an IL-2R include agents that

bind to IL-2 or an IL-2R as well as agents that bind to and subsequently destroy IL-2R-bearing cells, such as activated T cells. As described above in the context of IL-21 or IL-15 targeting, agents useful in achieving immune suppression can contain a moiety that targets the agent to an IL-2R-bearing cell and a target-cell depleting (*e.g.*, lytic) moiety that leads to the elimination of the IL-2R-bearing cell. For example, the targeting moiety can bind an IL-2R without effectively transducing a signal through that receptor. In the event an Fc region is included, that region can be derived from the same immunoglobulin molecules described above.

Targeting agents such as an IL-2/Fc agent, which acts as an IL-2 agonist (*e.g.*, *see* Zheng *et al.*, *J. Immunol.* 163:4041-4048, 1999; Rabinovitch *et al.*, *Diabetes* 51:638-645, 2002), can be administered with an agent that prevents IL-2-mediated IL-2R signaling, such as rapamycin or any of the agents described herein that inhibit cellular proliferation. Such agents are well known to those of ordinary skill in the art.

Instead of using, or in addition to using, an IL-2R targeting polypeptide (*e.g.*, an IL-2 polypeptide), the therapeutic agent used in combination with an IL-21 or an IL-15 antagonist can be an anti-IL-2 or an anti-IL-2R antibody (*e.g.*, a humanized antibody) that antagonizes IL-2 or the IL-2R, respectively.

As explained above, the methods of the invention (*e.g.*, methods of inhibiting an immune response (*e.g.*, a cellular immune response), methods of inhibiting transplant rejection, and methods of treating cancer) can also be carried out with compositions (*e.g.*, pharmaceutically acceptable compositions) that contain: (a) two or more agents, each of which promote T cell death or (b) at least one agent that promotes T cell death and at least one agent that inhibits T cell proliferation. The agent that promotes T cell death can do so by promoting AICD (activation induced cell death), and such agents include IL-2 and molecules that function as IL-2 agonists. For example, IL-2/Fc, mutants of IL-2 that retain the ability to bind and transduce a signal through the IL-2 receptor, and antibodies that specifically bind and agonize the IL-2 receptor (*e.g.*, an antibody that specifically binds the α subunit of the IL-2 receptor) can be included in the compositions of the invention. Other agents that promote AICD include Fas Ligand (FasL), which stimulates T cell death by activating the Fas signal transduction cascade on activated T cells, and biologically active mutants thereof.

Agents that Promote Passive Cell Death

Passive T cell death occurs when a T cell is deprived of an agent that is required for its survival. In addition to IL-15, factors including IL-4, IL-7, OX-40 ligand, IFNβ, 4-1BB and IGF-I are essential (*i.e.*, T cells die in the absence of each of these factors; *see, e.g.*, Tu *et al., J. Immunol.* 165:1331-1336, 2000; Tsuda *et al., J. Immunol. Meth.* 236:37-51, 2000; Bertolino *et al., Int. Immunol.* 11:1225-1238, 1999; Takahashi *et al., J. Immunol.* 162:5037-5040, 1999; Pilling *et al., Eur. J. Immunol.* 29:1041-1050, 1999; Chu *et al., J. Immunol.* 162:1896-1903, 1999; and Weinberg *et al., Semin. Immunol.* 10:471-480, 1998). One can deprive T cells of one or more of these factors (IL-15, IL-4, IL-7, *etc.*) by, for example, exposing the cells, *in vivo* or in culture, to agents that selectively bind to one or more of the factors or otherwise prevent them from interacting with the T cell as they normally would (the result of the deprivation being passive cell death). Alternatively, or in addition, one can inhibit the expression of nucleic acids encoding these factors.

Agents that Promote ADCC or CDC

ADCC and CDC can be provoked by agents that bind to the T cell surface and that contain an Fc portion of an immunoglobulin molecule that activates ADCC or CDC. Examples of such agents include antibodies that bind to cell surface structures that are expressed on activated immune cells (*e.g.*, cell surface receptors such as CD154, the IL-21 receptor, the IL-2 receptor, and the IL-15 receptor). In addition, one can use a ligand/Fc chimeric fusion protein, which binds to receptor proteins on the surface of activated cells (*e.g.*, a mutant IL-21/Fc, an IL-2/Fc or a mutant IL-15/Fc). Given these examples, other suitable agents will be apparent to those of ordinary skill in the art.

Agents that Inhibit Cellular Proliferation

Agents that inhibit cellular proliferation include rapamycin (Sirolimus), mycophenolate mofetil (MMF), azathioprine, and any other of the agents that are known to be useful for the treatment of hyperproliferative disorders (such as cancer). Well-characterized chemotherapeutics include agents that inhibit nucleic acid

metabolism (such as purine and pyrimidine biosynthesis inhibitors, RNA synthesis inhibitors, and DNA binding, DNA modifying, or intercalating agents). These agents are especially useful when the composition used to, for example, inhibit an immune response, also contains an agent such as IL-2/Fc, which not only promotes AICD but also stimulates T cell proliferation.

Agents that inhibit cellular proliferation also include folic acid antimetabolites such as methotrexate (MTX) and pyrimethamine; purine antimetabolites (such as 6-mercaptopurine (6-MP) and azathioprine) and pyrimidine antagonists such as cytarabine (ara-C), 5-azacytidine, and 5-fluorouracil (these categories were mentioned above); alkylating and other DNA-linking agents (*e.g.*, cyclophosphamide (CPA); mitomycin C, and Doxorubicin (Adriamycin)); vinca alkaloids (*e.g.*, vincristine); and calcineurin inhibitors (*e.g.*, Cyclosporin A, FK506, and Brequinar).

Other agents that can be used to inhibit cellular proliferation include agents that interfere directly with proteins involved in cell cycle regulation (such as anti-CDKs (Cell Division Kinase) or anti-cyclins) or proteins that affect cell proliferation check points (all proliferating cells have check points at different stages of the cell cycle that prevent them from entering the next stage of the cell division cycle (CDC) before they have concluded the previous step). Pathways that feed into check point controls include DNA-, RNA- and protein-synthesis inhibitors (*e.g.*, S6 kinase and PI-3-kinase inhibitors). Cytokinesis inhibitors can also be used.

Procedures for Screening Agents that Inhibit the Immune Response

In addition to testing a candidate agent (*e.g.*, a mutant IL-21, mutant IL-15 or IL-2 polypeptide) in *in vitro* assays (as described, for example, in U.S. Patent No. 6,001,973) one can use any of the following *in vivo* assays to test which particular combinations of the agents described herein most effectively bring about immune suppression. For example, one can test one or more of the agents that target the IL-21R or the IL-15R in combination with one or more of the agents that antagonize IL-2 or its receptor. These *in vivo* assays represent only some of the routine ways in which one of ordinary skill in the art could further test the efficacy of agents of the invention. They were selected for inclusion here because of their relevance to the variety of clinical conditions amenable to treatment with agents that target IL-21,

IL-15, IL-2, and their receptors. For example, the assays are relevant to organ transplantation, immune disease, particularly autoimmune disease, graft versus host disease and cancers of the immune system (*e.g.* cancers that arise when T cells become malignant).

Transplantation Paradigms

To determine whether a combination of agents of the invention achieves immune suppression, the combination can be administered (either directly, by gene-based therapy, or by cell-based therapy) in the context of well-established transplantation paradigms.

Agents of the invention, nucleic acid molecules encoding them (or that hybridize with and thereby inhibit them), can be systemically or locally administered by standard means to any conventional laboratory animal, such as a rat, mouse, rabbit, guinea pig, or dog, before an allogeneic or xenogeneic skin graft, organ transplant, or cell implantation is performed on the animal. Strains of mice such as C57B1-10, B10.BR, and B10.AKM (Jackson Laboratory, Bar Harbor, ME), which have the same genetic background but are mismatched for the H-2 locus, are well suited for assessing various organ grafts.

Heart Transplantation

A method for performing cardiac grafts by anastomosis of the donor heart to the great vessels in the abdomen of the host was first published by Ono *et al.* (*J. Thorac. Cardiovasc. Surg.* 57:225, 1969; see also Corry *et al.*, *Transplantation* 16:343, 1973). By way of this surgical procedure, the aorta of a donor heart is anastomosed to the abdominal aorta of the host, and the pulmonary artery of the donor heart is anastomosed to the adjacent vena cava using standard microvascular techniques. Once the heart is grafted in place and warmed to 37°C with Ringer's lactate solution, normal sinus rhythm will resume. Function of the transplanted heart can be assessed frequently by palpation of ventricular contractions through the abdominal wall. Rejection is defined as the cessation of myocardial contractions. Agents of the invention (*e.g.*, a combination of mutant IL-21/Fc and/or mutant IL-15/Fc and an antibody that binds to and inhibits IL-2 or IL-2R, or a combination of

a mutant IL-15/FC, IL-2/Fc, and rapamycin) would be considered effective in reducing organ rejection if hosts that received these agents experienced a longer period of engraftment of the donor heart than did untreated hosts.

Skin Grafting

The effectiveness of various combinations of the agents of the invention can also be assessed following a skin graft. To perform skin grafts on a rodent, a donor animal is anesthetized and the full thickness skin is removed from a part of the tail. The recipient animal is also anesthetized, and a graft bed is prepared by removing a patch of skin from the shaved flank. Generally, the patch is approximately 0.5 x 0.5 cm. The skin from the donor is shaped to fit the graft bed, positioned, covered with gauze, and bandaged. The grafts can be inspected daily beginning on the sixth post-operative day, and are considered rejected when more than half of the transplanted epithelium appears to be non-viable. Agents of the invention (*e.g.*, a combination of mutant IL-21/Fc and/or mutant IL-15/Fc and an antibody that binds to and inhibits IL-2 or IL-2R, or a combination of a mutant IL-15/FC, IL-2/Fc, and rapamycin) would be considered effective in reducing skin graft rejection if hosts that received these agents experienced a longer period of engraftment of the donor skin than did untreated hosts.

Islet Allograft Model

DBA/2J islet cell allografts can be transplanted into rodents, such as 6-8 week-old B6 AF1 mice rendered diabetic by a single intraperitoneal injection of streptozotocin (225 mg/kg; Sigma Chemical Co., St. Louis, MO). As a control, syngeneic islet cell grafts can be transplanted into diabetic mice. Islet cell transplantation can be performed by following published protocols (for example, see Gotoh *et al.*, *Transplantation* 42:387, 1986). Briefly, donor pancreata are perfused *in situ* with type IV collagenase (2 mg/ml; Worthington Biochemical Corp., Freehold, NJ). After a 40-minute digestion period at 37°C, the islets are isolated on a discontinuous Ficoll gradient. Subsequently, 300-400 islets are transplanted under the renal capsule of each recipient. Allograft function can be followed by serial blood glucose measurements (Accu-Check III™; Boehringer, Mannheim, Germany).

Primary graft function is defined as a blood glucose level under 11.1 mmol/l on day 3 post-transplantation, and graft rejection is defined as a rise in blood glucose exceeding 16.5 mmol/l (on each of at least two successive days) following a period of primary graft function.

Models of Autoimmune Disease

Models of autoimmune disease provide another means to assess combinations of the agents of the invention *in vivo*. These models are well known to those of ordinary skill in the art and can be used to determine whether a given combination of agents, which includes, for example, an agent that targets an IL-21R, would be therapeutically useful in treating a specific autoimmune disease when delivered either directly, via genetic therapy, or via cell-based therapies.

Autoimmune diseases that have been modeled in animals include rheumatic diseases, such as rheumatoid arthritis and systemic lupus erythematosus (SLE), type I diabetes, and autoimmune diseases of the thyroid, gut, and central nervous system. For example, animal models of SLE include MRL mice, BXSB mice, and NZB mice and their F_1 hybrids. These animals can be crossed in order to study particular aspects of the rheumatic disease process; progeny of the NZB strain develop severe lupus glomerulonephritis when crossed with NZW mice (Bielschowsky *et al.*, *Proc. Univ. Otago Med. Sch.* 37:9, 1959; see also *Fundamental Immunology*, Paul, Ed., Raven Press, New York, NY, 1989). Similarly, a shift to lethal nephritis is seen in the progeny of NBZ X SWR matings (Data *et al.*, *Nature* 263:412, 1976). The histological appearance of renal lesions in SNF_1 mice has been well characterized (Eastcott *et al.*, *J. Immunol.* 131:2232, 1983; see also *Fundamental Immunology, supra*). Therefore, the general health of the animal as well as the histological appearance of renal tissue can be used to determine whether the administration of agents that target an IL-21R and, *e.g.*, target the IL-15R, can effectively suppress the immune response in an animal model of SLE.

One of the MRL strains of mice that develops SLE, MRL-*lpr/lpr*, also develops a form of arthritis that resembles rheumatoid arthritis in humans (Theofilopoulos *et al.*, *Adv. Immunol.* 37:269, 1985). Alternatively, an experimental arthritis can be induced in rodents by injecting rat type II collagen (2 mg/ml) mixed

1:1 in Freund's complete adjuvant (100 μl total) into the base of the tail. Arthritis develops 2-3 weeks after immunization.

An experimental arthritis can also be induced in DBA/1J mice by injecting bovine type II collagen (CII), as follows. DBA/1J mice are immunized on day 1 with 100 μl of solution containing 100 μg of bovine CII in CFA, injected intradermally at the based of the tail. CII is dissolved overnight in 5 ml of PBS under constant agitation in a cold room. On the day of immunization, dissolved CII (5 ml) is emulsified with 2.5 ml of Freund's complete adjuvant and 2.5 ml of Freund's incomplete adjuvant. The final concentration of *mycobacterium tuberculosis* is 1 mg/ml. On day 21, all the animals receive a booster immunization of 100 μl of 100 μg of CII in Freund's incomplete adjuvant intradermally at the base of the tail. CII is dissolved as described above and emulsified in a 1:1 volume ratio with Freund's incomplete adjuvant. Mice are scored every day for signs of arthritis from day 21 to the end of the experiment. At the first sign(s) of arthritis, mice are randomly incorporated in a control group (*e.g.*, no treatment) or a treatment group (*e.g.*, mIL-15/Fc). Treatment is carried out for 14 days and the efficacy of the treatment is monitored by disease symptom development, histology and gene expression analysis.

The ability of nucleic acid molecules encoding agents of the invention (*e.g.*, agents that target the IL-21R and agents that target the Il-15R or that bind to and inactivate antigen-activated T cells) to suppress an immune response can be assessed by targeting the nucleic acid molecules to T lymphocytes. One way to target T lymphocytes is as follows. Spleen cell suspensions are prepared 2-3 days after the onset of arthritis and incubated with collagen (100 μg/ml) for 48 hours to induce proliferation of collagen-activated T cells. During this time, the cells are transduced with a vector encoding the polypeptide agent of interest. As a control, parallel cultures are grown but not transduced or, transduced with an "empty" vector. The cells are then injected intraperitoneally (5×10^7 cells/animal). The effectiveness of the treatment is assessed by following the disease symptoms during the subsequent 2 weeks, as described by Chernajovsky *et al.* (*Gene Therapy* 2:731-735, 1995). Lesser symptoms, compared to control, indicate that the combined agents of the invention, and the nucleic acid molecules that encode them, function as

immunosuppressants and are therefore useful in the treatment of immune disease, particularly autoimmune disease.

The ability of various combinations of agents to suppress the immune response in the case of Type I diabetes can be tested in the BB rat strain, which was developed from a commercial colony of Wistar rats at the Bio-Breeding Laboratories in Ottawa. These rats spontaneously develop autoantibodies against islet cells and insulin, just as occurs with human Type I diabetes. Alternatively, NOD (non-obese diabetic) mice can be used as a model system. Autoimmune diseases of the thyroid have been modeled in the chicken. Obese strain (OS) chickens consistently develop spontaneous autoimmune thyroiditis resembling Hashimoto's disease (Cole *et al.*, *Science* 160:1357, 1968). Approximately 15% of these birds produce autoantibodies to parietal cells of the stomach, just as in the human counterpart of autoimmune thyroiditis. The manifestations of the disease in OS chickens, which could be monitored in the course of any treatment regime, include body size, fat deposit, serum lipids, cold sensitivity, and infertility.

Models of autoimmune disease in the central nervous system (CNS) can also be experimentally induced. An inflammation of the CNS, which leads to paralysis, can be induced by a single injection of brain or spinal cord tissue with adjuvant in many different laboratory animals, including rodents and primates. This model, referred to as experimental allergic encephalomyelitis (EAE) is T cell mediated. Similarly, experimentally induced myasthenia gravis can be produced by a single injection of acetylcholine receptor with adjuvants (Lennon *et al.*, *Ann. N.Y. Acad. Sci.* 274:283, 1976).

Autoimmune diseases of the gut can be modeled in IL-2 or IL-10 "knock out" mice, or in mice that receive enemas containing bovine serum albumin.

Nucleic Acid Molecules That Encode Agents of the Invention

Polypeptide agents of the invention, including those that are fusion proteins (*e.g.*, the mutant IL-21/Fc, mutant IL-15/Fc and IL-2/Fc molecules) can not only be obtained by expression of a nucleic acid molecule in a suitable eukaryotic or prokaryotic expression system *in vitro* and subsequent purification of the polypeptide agent, but can also be administered to a patient by way of a suitable gene therapeutic

expression vector encoding a nucleic acid molecule. Furthermore, a nucleic acid can be introduced into a cell of a graft prior to transplantation of the graft. Thus, nucleic acid molecules encoding the agents described above are within the scope of the invention.

The nucleic acid molecules that encode agents of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In addition, the nucleic acid molecules can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand).

The nucleic acid molecules of the invention are referred to as "isolated" because they are separated from either the 5' or the 3' coding sequence with which they are immediately contiguous in the naturally occurring genome of an organism. Thus, the nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding sequence can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction endonucleases, or by performance of the polymerase chain reaction (PCR). In the event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced by *in vitro* transcription.

The isolated nucleic acid molecules of the invention can include fragments not found as such in the natural state. Thus, the invention encompasses recombinant molecules, such as those in which a nucleic acid sequence (for example, a sequence encoding a mutant IL-21 or mutant IL-15) is incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location).

As described above, agents of the invention can be fusion proteins. In addition to, or in place of, the heterologous polypeptides described above, a nucleic acid molecule encoding an agent of the invention can contain sequences encoding a

"marker" or "reporter." Examples of marker or reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, one of ordinary skill in the art will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter.

The nucleic acid molecules of the invention can be obtained by introducing a mutation into an agent of the invention (*e.g.*, an IL-21 molecule, an IL-15 molecule, or an IL-2 molecule) obtained from any biological cell, such as the cell of a mammal, or produced by routine cloning methods. Thus, the nucleic acids of the invention can be those of a mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, baboon, dog, or cat. Preferably, the nucleic acid molecules will be those of a human.

The nucleic acid molecules described above can be contained within a vector that is capable of directing their expression in, for example, a cell that has been transduced with the vector. Accordingly, in addition to polypeptide agents, expression vectors containing a nucleic acid molecule encoding those agents and cells transfected with those vectors are among the preferred embodiments.

Vectors suitable for use in the present invention include T7-based vectors for use in bacteria (*see, e.g.,* Rosenberg *et al.*, *Gene* 56:125, 1987), the pMSXND expression vector for use in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988), yeast expression systems, such as Pichia pastoris (for example the PICZ family of expression vectors from Invitrogen, Carlsbad, CA) and baculovirus-derived vectors (for example the expression vector pBacPAK9 from Clontech, Palo Alto, CA) for use in insect cells. The nucleic acid inserts, which encode the polypeptide of interest in such vectors, can be operably linked to a promoter, which is selected based on, for example, the cell type in which expression is sought. For example, a T7 promoter can be used in bacteria, a polyhedrin promoter can be used in insect cells, and a cytomegalovirus or metallothionein promoter can be used in mammalian cells. Also, in the case of higher eukaryotes, tissue-specific and cell type-specific promoters are widely available. These promoters are so named for

their ability to direct expression of a nucleic acid molecule in a given tissue or cell type within the body. One of ordinary skill in the art is well aware of numerous promoters and other regulatory elements that can be used to direct expression of nucleic acids.

In addition to sequences that facilitate transcription of the inserted nucleic acid molecule, vectors can contain origins of replication, and other genes that encode a selectable marker. For example, the neomycin-resistance (*neo*^{r}) gene imparts G418 resistance to cells in which it is expressed, and thus permits phenotypic selection of the transfected cells. Other feasible selectable marker genes allowing for phenotypic selection of cells include various fluorescent proteins, e.g. green fluorescent protein (GFP) and variants thereof. Those of skill in the art can readily determine whether a given regulatory element or selectable marker is suitable for use in a particular experimental context.

Viral vectors that can be used in the invention include, for example, retroviral, adenoviral, and adeno-associated vectors, herpes virus, simian virus 40 (SV40), and bovine papilloma virus vectors (*see, e.g.*, Gluzman (Ed.), *Eukaryotic Viral Vectors*, CSH Laboratory Press, Cold Spring Harbor, New York).

Prokaryotic or eukaryotic cells that contain a nucleic acid molecule that encodes an agent of the invention and express the protein encoded in that nucleic acid molecule *in vitro* are also features of the invention. A cell of the invention is a transfected cell, *i.e.*, a cell into which a nucleic acid molecule, for example a nucleic acid molecule encoding a mutant IL-21 polypeptide, has been introduced by means of recombinant DNA techniques. The progeny of such a cell are also considered within the scope of the invention. The precise components of the expression system are not critical. For example, a mutant IL-21 polypeptide can be produced in a prokaryotic host, such as the bacterium *E. coli*, or in a eukaryotic host, such as an insect cell (for example, Sf21 cells), or mammalian cells (*e.g.*, COS cells, CHO cells, 293 cells, NIH 3T3 cells, or HeLa cells). These cells are available from many sources, including the American Type Culture Collection (Manassas, VA). In selecting an expression system, it matters only that the components are compatible with one another. One of ordinary skill in the art is able to make such a determination. Furthermore, if guidance is required in selecting an expression system, one can

consult Ausubel *et al.* (*Current Protocols in Molecular Biology*, John Wiley and Sons, New York, NY, 1993) and Pouwels *et al.* (*Cloning Vectors: A Laboratory Manual*, 1985 Suppl. 1987).

Eukaryotic cells that contain a nucleic acid molecule that encodes the agent of the invention and express the protein encoded in such nucleic acid molecule *in vivo* are also features of the invention.

Furthermore, eukaryotic cells of the invention can be cells that are part of a cellular transplant, a tissue or organ transplant. Such transplants can comprise either primary cells taken from a donor organism or cells that were cultured, modified and/or selected *in vitro* before transplantation to a recipient organism (*e.g.*, eurkaryotic cells lines, including stem cells or progenitor cells). Since, after transplantation into a recipient organism, cellular proliferation may occur, the progeny of such a cell are also considered within the scope of the invention. A cell, being part of a cellular, tissue or organ transplant, can be transfected with a nucleic acid encoding a mutant IL-21 or mutant IL-15 polypeptide and subsequently be transplanted into the recipient organism, where expression of the mutant IL-21 or the mutant IL-15 polypeptide occurs. Furthermore, such a cell can contain one or more additional nucleic acid constructs allowing for application of selection procedures (*e.g.*, selection of specific cell lineages or cell types prior to transplantation into a recipient organism).

The expressed polypeptides can be purified from the expression system using routine biochemical procedures, and can be used as diagnostic tools or as therapeutic agents, as described below.

Agents that Target an IL-21R or an IL-15R are Useful in Making Diagnoses

Agents that target an IL-21R or an IL-15R can be used to determine whether a patient has a disease (*e.g.*, an immune disease, particularly an autoimmune disease) that is amenable to treatment with a combination of the agents described herein. The diagnostic method can be carried out, for example, by obtaining a sample of tissue from a patient suspected of having an immune disease, particularly autoimmune disease or a cancer that is manifest as malignant immune cells and exposing that

tissue to an antigenically-tagged polypeptide that targets an IL-21R or an IL-15R. The sample may be any biological sample, such as a blood, urine, serum, or plasma sample. In addition, the sample may be a tissue sample (*e.g.*, biopsy tissue), or an effusion obtained from a joint (*e.g.*, synovial fluid), from the abdominal cavity (*e.g.*, ascites fluid), from the chest (*e.g.*, pleural fluid), or from the central nervous system (*e.g.*, cerebral spinal fluid). The sample may also consist of cultured cells that were originally obtained from a patient (*e.g.*, peripheral blood mononuclear cells). The sample can be obtained from a mammal, such as a human patient. If the sample contains cells that are bound by the agent to which they are exposed, it is highly likely that they would be bound by that agent (*e.g.* an agent that targets an IL-15R) *in vivo* and could thereby be inhibited from proliferating or destroyed *in vivo*. The presenting symptoms of candidate patients for such testing and the relevant tissues to be sampled given a particular set of symptoms are well known to one of ordinary skill in the art.

Patients Amenable to Treatment

The compositions of the invention are useful in inhibiting T cells that are involved, or would be involved, in an immune response (*e.g.*, a cellular immune response) to an antigen; in inhibiting other cells involved in the pathogenesis of immunological disorders (*e.g.*, monocytes, macrophages, and other antigen presenting cells such as dendritinc cells, NK cells, and granulocytes); and in destroying hyperproliferating cells (as seen, for example, in tissues involved in immunological disorders such as synovial fibroblasts (which are affected in rheumatoid arthritis) keratinocytes (which are affected in psoriasis), or dermal fibroblases (which are affected in systemic lupus erythematosis). Given these examples, other cell types that can usefully be targeted will be apparent to those of ordinary skill in the art. Hyperproliferative cells may also be cancerous cells (*e.g.*, malignant T cells).

Thus, the compositions of the invention can be used to treat patients who are suffering from an immune disease, particularly autoimmune disease, including but not limited to the following: (1) a rheumatic disease such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome or Behcet's disease (2) type I or type II diabetes (3) an autoimmune disease of the thyroid, such as

Hashimoto's thyroiditis or Graves' Disease (4) an autoimmune disease of the central nervous system, such as multiple sclerosis, myasthenia gravis, or encephalomyelitis (5) a variety of phemphigus, such as phemphigus vulgaris, phemphigus vegetans, phemphigus foliaceus, Senear-Usher syndrome, or Brazilian phemphigus, (6) diseases of the skin such as psoriasis or neurodermitis, and (7) inflammatory bowel disease (*e.g.*, ulcerative colitis or Crohn's Disease). Combinations of the agents of the invention can also be used to treat acquired immune deficiency syndrome (AIDS). Similarly, methods by which these agents are administered can be used to treat a patient who has received a transplant of synthetic or biological material, or a combination of both. Such transplants can be organ, tissue or cell transplants, or synthetic grafts seeded with cells, for example, synthetic vascular grafts seeded with vascular cells. In addition, patients suffering from GVHD or patients who have received a vascular injury would benefit from this method.

Because the invention encompasses administration of a target-cell depleting form of an agent that targets the IL-21R or the IL-15R (or an IL-2 receptor, or a combination of IL-21, IL-15 or IL-2 (or their receptors)), it is possible to selectively kill autoreactive or "transplant destructive" immune cells without massive destruction of normal T cells. Accordingly, the invention features a method of killing cells that express the IL-21R *in vivo,* which includes activated or autoreactive or "transplant destructive" immune cells or malignant cells. These methods can be carried out by administering to a patient a combination of agents that includes an agent that targets the IL-21R and that activates the complement system, lyses cells by the ADCC mechanism, or otherwise kills cells expressing the wild-type IL-15 receptor complex.

Formulations for Use and Routes of Administration

The methods of the present invention and the therapeutic compositions used to carry them out contain "substantially pure" agents. For example, in the event the agent is a polypeptide, the polypeptide is at least 60% by weight (dry weight) the polypeptide of interest, *e.g.*, a polypeptide that binds and destroys IL-21R-bearing cells or IL-15R-bearing cells. Preferably, the agents (*e.g.*, the polypeptides) are at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight,

the agent of interest. Purity can be measured by any appropriate standard method, *e.g.*, column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

Although agents useful in the methods of the present invention can be obtained from naturally occurring sources, they can also be synthesized or otherwise manufactured (*e.g.*, agents that bind and destroy IL-21R-bearing cells or IL-15R-bearing cells can be produced by expression of a recombinant nucleic acid molecule). Polypeptides that are derived from eukaryotic organisms or synthesized in *E. coli*, or other prokaryotes, and polypeptides that are chemically synthesized will be substantially free from their naturally associated components. In the event the polypeptide is a chimera, it can be encoded by a hybrid nucleic acid molecule containing one sequence that encodes all or part of the agent (*e.g.*, a sequence encoding a mutant IL-21 polypeptide and sequence encoding an Fc region of IgG). Agents of the invention (*e.g.*, polypeptides) can be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein, or to a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

The techniques that are required to make the agents of the invention are routine in the art, and can be performed without resort to undue experimentation by one of ordinary skill in the art. For example, a mutation that consists of a substitution of one or more of the amino acid residues in IL-21 or IL-15 can be created using the PCR-assisted mutagenesis technique described herein for the creation of the mutant IL-15 polypeptide in which glutamine residues at positions 149 and 156 were changed to aspartic acid residues or for the creation of a mutant IL-21 polypeptide in which glutamine residues at positions 119 and 114 were changed to aspartic acid residues. Mutations that consist of deletions or additions of amino acid residues (to an IL-21 or IL-15 polypeptide or to any of the other useful polypeptides described herein, *e.g.*, polypeptides that inhibit costimulation or that bind activated T cells) can also be made with standard recombinant techniques. In therapeutic applications, agents of the invention can be administered with a physiologically acceptable carrier, such as physiological saline. The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to one of ordinary skill in the art. Excipients that can be used include buffers (*e.g.*, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid,

phospholipids, proteins (*e.g.*, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The agents of the invention can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences."

Routes of administration are also well known to skilled pharmacologists and physicians and include intraperitoneal, intramuscular, subcutaneous, and intravenous administration. Additional routes include intracranial (*e.g.*, intracisternal or intraventricular), intraorbital, opthalmic, intracapsular, intraspinal, intraperitoneal, transmucosal, topical, subcutaneous, and oral administration. It is expected that the intravenous or intra-arterial routes will be preferred for the administration of agents that target an IL-21 receptor. The subcutaneous route represents another preferred mode of administration as the subcutaneous tissue provides a stable environment for polypeptides, from which they can be slowly released.

In case of cell-based therapies (gene therapies), the cells/tissues/organs could either be transfected by incubation, infusion or perfusion prior to transplantation with a nucleic acid composition, such that the therapeutic protein is expressed and subsequently released by the transplanted cells/tissues/organs within the recipient organism. As well, the cells/tissues/organs could undergo a pretreatment by perfusion or simple incubation with the therapeutic protein prior to transplantation in order to eliminate transplant-associated immune cells adherent to the donor cells/tissues/organs (although this is only a side aspect, which will probably not be of any clinical relevance). In the case of cell transplants, the cells may be administered either by an implantation procedure or with a catheter-mediated injection procedure through the blood vessel wall. In some cases, the cells may be administered by release into the vasculature, from which the subsequently are distributed by the blood stream and/or migrate into the surrounding tissue (this is done in islet cells transplantation, where the islet cells are released into the portal vein and subsequently migrate into liver tissue).

It is well known in the medical arts that dosages for any one patient depend on many factors, including the general health, sex, weight, body surface area, and age of the patient, as well as the particular compound to be administered, the time and route of administration, and other drugs being administered concurrently. Dosages for the polypeptide of the invention will vary, but can, when administered intravenously, be given in doses on the order of magnitude of 1 microgram to 10 mg/kg body weight or on the order of magnitude of 0.01 mg/l to 100 mg/l of blood volume. A dosage can be administered one or more times per day, if necessary, and treatment can be continued for prolonged periods of time. Determining the correct dosage for a given application is well within the abilities of one of ordinary skill in the art.

EXAMPLES

Creation of an IL-21/Fc fusion protein

Full-length, mature human and murine IL-21 polypeptides were fused at their C-termini with the hinge, CH2, and CH3 domains or either human IgG1 or murine IgG2a, respectively, to produce either a human IL-21/human IgG1 Fc fusion protein or a murine IL-21/murine IgG2a Fc chimeric protein.

Human IL-21/Fc is capable of transducing signals through the IL-21 receptor, has an increased *in vivo* serum half-life and can activate complement mediated lysis or antibody-dependent cellular cytotoxicity. Similarly, murine IL-21/Fc is capable of transducing signals through the murine IL-21 receptor, has an increased *in vivo* serum half-life and can activate complement mediated lysis or antibody-dependent cellular cytotoxicity.

Identification of a novel amino acid residue at the C terminus of human and murine IL-21 that is important for interaction with the IL-21 receptor: Alignment of IL-21, -15, and -2

A glutamine (Q) residue that is critical for the interaction of IL-2 and IL-15 with the common gamma chain of the IL-2 and IL-15 receptors has been identified at position 119 (of the mature protein) in human and murine IL-21. A second glutamine at position 114 of mature IL-21 is also conserved between human and murine IL-21 and IL-15.

Creation of a cytolytic mutant IL-12/Fc fusion protein

Full-length mature human or murine IL-21 carrying point mutations at either amino acid residues 119 (Gln to Asp), 114 (Gln to Asp) or at both amino acid positions were fused at their C-termini with the hinge, CH2, and CH3 domains of either human IgG1 or murine IgG2a, respectively, to produce either a human mutant IL-21/human IgG1 Fc or a murine mutant IL-21/murine IgG2a Fc chimeric protein. Mutant IL-21/Fc is capable of binding to the IL-21 receptor at high affinity but does not transduce a signal through the IL-21 receptor, thereby effectively antagonizing IL-21 activity on target cells. Furthermore, mIL-21/Fc has an increased *in vivo* serum half-life and can activate complement mediated lysis or antibody-dependent cellular cytotoxicity.

Mutant IL-21/Fc inhibits anti-CD3 mediated human PBMC proliferation

Whereas IL-21/Fc co-stimulates the proliferation of anti-CD3 activated peripheral blood mononuclear cell (PBMC) proliferation, even at low concentrations, mutant IL-21/Fc inhibits the proliferation of PBMC stimulated with anti-CD3 in a dose-dependent manner.

Human PBMC were isolated by Ficoll-Paque gradients from whole blood obtained from healthy volunteers. The PBMC (5×10^5/well) were cultured in 96-well flat-bottomed plates for three days in the presence of 0.25 μg/ml soluble anti-human CD3 monoclonal antibody. Cell proliferation was monitored by adding 1 μCi ^{3}H-thymidine/well 12 hours prior to harvesting. Human IL-21/Fc and mutant human IL-21/Fc (double mutant) were added to the cells for the duration of the culture at decreasing concentrations using 1:1 serial dilutions. The highest concentration of IL-21/Fc and mutant IL-21/Fc used was 50 μg/ml (= log2). The results are shown in Fig. 7. The mutant IL-21/Fc, but not wild type IL-21/Fc blocked anti-CD3 triggered PBMC proliferation *in vitro*.

Mutant IL-21/Fc blocks not only IL-21-mediated, but also IL-2-mediated co-stimulation of proliferation of anti-CD3 activated PBMC.

Whereas IL-2 or IL-2 plus IL-21/Fc enhance the proliferation of anti-CD3 activated PBMC in a dose-dependent manner, the addition of mutant IL-21/Fc inhibits not only the proliferation of PBMC cultured in the presence of anti-CD3 plus IL-21/Fc, but also of PBMC cultured in the presence of either anti-CD3 plus IL-2 or anti-CD3 plus IL-2 plus IL-21/Fc.

Human PBMC were isolated and cultured in the presence of anti-CD3, as described above. Recombinant human IL-21/Fc (5 μg/ml) and mutant IL-21/Fc (5 μg/ml) were added as indicated in the Figure. IL-2 was added to the cells for the duration of the culture at decreasing concentrations using 1:1 serial dilutions (100 U/ml highest concentration = log2). The results are shown in Fig. 8. Mutant IL-21/Fc blocks anti-CD3, IL-2, and IL-21 triggered PBMC proliferation *in vitro*.

Mutant IL-21/Fc blocks not only IL-21 mediated, but also IL-15 mediated co-stimulation of proliferation of anti-CD3 activated PBMC.

Whereas IL-15 or IL-15 plus IL-21/Fc enhance the proliferation of anti-CD3 activated PBMC, the addition of mutant IL-21/Fc inhibits the proliferation of PBMC cultured in the presence of anti-CD3 plus IL-15 or anti-CD3 plus IL-15 plus IL-21/Fc.

Human PBMC were isolated and cultured in the presence of anti-CD3 as described above. Recombinant human IL-21/Fc (5 μg/ml) and mutant IL-21/Fc (5 μg/ml) were added as indicated in the Figure. IL-15 was added to the cells for the duration of the culture at decreasing concentrations using 1:1 serial dilutions (1 ng/ml, highest concentration = log2). The results are shown in Fig. 9. Mutant IL-21/Fc blocks anti-CD3, IL-15 and IL-21 triggered PBMC proliferation *in vitro*.

Proliferation of PHA-Stimulated Human PBMCs

The ability of the FLAG-HMK-IL-15 double mutant polypeptide to bind PHA activated human PBMCs was demonstrated as follows. PHA-activated PBMCs were washed and incubated with medium alone, or with the FLAG-HMK-IL-15 double mutant. The cells were then incubated with an anti-FLAG biotinylated antibody and stained with streptavidin conjugated to RED670. The stained cells were analyzed by flow cytometry.

FACS Analysis of Leukemic Cell Lines Stained with Wild-Type FLAG-HMK-IL-15

In a series of experiments similar to those above, the ability of the wild-type FLAG-HMK-IL-15 polypeptide to bind leukemia cells was shown. The cells treated were obtained from the leukemic cell lines MOLT-14, YT, HuT-102, and from cell lines currently being established at Beth Israel Hospital (Boston, MA), and named 2A and 2B. The cultured cells were washed and incubated with either medium alone or with medium containing the FLAG-HMK-IL-15 wild-type polypeptide. The cells were then incubated with the biotinylated anti-FLAG antibody and stained with RED670-conjugated streptavidin. The stained cells were analyzed by flow cytometry.

Genetic Construction of Additional Mutant IL-15 Chimeric Polypeptides

In addition to the FLAG-HMK-IL-15 chimera, which provides the mutant IL-15 with an antigenic tag, numerous other polypeptides can be linked to any mutant of IL-15 (or IL-21 or IL-2). For example, mutant IL-21, mutant IL-15, or IL-2 can be linked to the Fc fragment of the IgG subclass of antibodies according to the following method.

Genetic Construction of Mutant IL-15/Fc

cDNA for Fcγ2a can be generated from mRNA extracted from an IgG2a secreting hybridoma using standard techniques with reverse transcriptase (MMLV-RT; Gibco-BRL, Grand Island, NY) and a synthetic oligo-dT (12-18) oligonucleotide (Gibco BRL). The mutant IL-15 cDNA can be amplified from a plasmid template by PCR using IL-15 specific synthetic oligonucleotides.

The 5' oligonucleotide is designed to insert a unique *NotI* restriction site 40 nucleotides 5' to the translational start codon, while the 3' oligonucleotide eliminates the termination codon and modifies the C-terminal Ser residue codon usage from AGC to TCG to accommodate the creation of a unique *BamHI* site at the mutant IL-15/Fc junction. Synthetic oligonucleotides used for the amplification of the Fcγ2a domain cDNA change the first codon of the hinge from Glu to Asp in order to create a unique *BamHI* site spanning the first codon of the hinge and introduce a unique *XbaI* site 3' to the termination codon.

The Fc fragment can be modified so that it is non-target-cell depleting, *i.e.*, not able to activate the complement system. To make the non-target-cell depleting mutant IL-15 construct (mIL-15/Fc), oligonucleotide site directed mutagenesis is used to replace the C'1q binding motif Glu318, Lys320, Lys322 with Ala residues. Similarly, Leu235 is replaced with Glu to inactivate the FcγR I binding site. Ligation of cytokine and Fc" components in the correct translational reading frame at the unique *BamHI* site yields a 1,236 basepair open reading frame encoding a single 411 amino acid polypeptide (including the 18 amino acid IL-15 signal peptide) with a total of 13 cysteine residues. The mature secreted homodimeric IL-15/Fc is predicted to have a total of up to eight intramolecular and three inter-heavy chain disulfide linkages and a molecular weight of approximately 85 kDa, exclusive of glycosylation.

Expression and Purification of mIL-15 Receptor Fc Fusion Proteins

Proper genetic construction of mIL-15/Fc can be confirmed by DNA sequence analysis following cloning of the fusion gene as a *NotI-XbaI* cassette into the eukaryotic expression plasmid pRc/CMV (Invitrogen, San Diego, CA). Other constructs of the invention can be similarly confirmed. The plasmid pRc/CMV carries a CMV promoter/enhancer, a bovine growth hormone polyadenylation signal and a neomycin resistance gene for selection with G418. Plasmids carrying the mIL-15/Fc fusion gene is transfected into Chinese hamster ovary cells (CHO-K1, available from the American Type Culture Collection) by electroporation (1.5 kV/3 μF/0.4 cm/PBS) and selected in serum-free Ultra-CHO media (BioWhittaker Inc., Walkerville, MD) containing 1.5 mg/ml of G418 (Geneticin, Gibco BRL). After subcloning, clones that produce high levels of the fusion protein are selected by screening supernatants from IL-15 by ELISA (PharMingen, San Diego, CA). mIL-15/Fc fusion proteins are purified from culture supernatants by protein A sepharose affinity chromatography followed by dialysis against PBS and 0.22 μm filter sterilization. Purified proteins can be stored at -20°C before use.

Western blot analysis following SDS-PAGE under reducing (with DTT) and non-reducing (without DTT) conditions can be performed using monoclonal or polyclonal anti-mIL-15 or anti Fcγ primary antibodies to evaluate the size and isotype specificity of the fusion proteins. The functional activity of mutant IL-15/Fc can be assessed by a standard T cell proliferation assay, as described above. The following mAbs were obtained from PharMingen (San Diego, CA): PE-anti-mouse CD25 (IL-2R α chain, IgG1, PC61), rat anti-mouse CD122 (IL-2R β chain, IgG2b, TM-b1), rat anti-mouse CD132 (IL-2R γc, IgG2b, TUGm2), hamster anti-mouse CD3 (IgG, 145-2C11), hamster anti-mouse CD28 (IgG, 37.51), PE-anti-mouse CD62L (IgG2a, MEL14), PE conjugated hamster anti-mouse Bcl-2 (IgG, 3F11), PE conjugated anti-mouse IL-2 (IgG2b, JES6-5H4), PE-annexin V, biotinylated anti-rat IgG2b, PE-streptoavidin, PE-CyChrome, and PE conjugated isotype control mAbs. A biotinylated mouse anti-FLAG mAb and a rat IgG1 control mAb were obtained from Sigma Chemical Co. (St Louis, MO). A B-cell hybridoma secreting rat anti-mouse CD25 mAb (TIB 222, IgG1) was obtained from the American Type Culture Collection (ATCC; Manassas, VA). Cells were grown in serum free UltraCulture

medium (BioWhittaker, Walkerville, MD) and the mAb in the culture supernatant was purified with a protein G column.

Expression Studies of IL-2 and IL-15 *in vivo*.

Recombinant human IL-2 and IL-15 were purchased from R & D System (Minneapolis, MN). IL-15-FLAG and IL-15 mutant/Fc fusion proteins were constructed, expressed, and tested as previously reported (Chae *et al.*, *J. Immunol.* 157:2813-2819, 1996; Kim *et al.*, *J. Immunol.* 161:5742-5748, 1998). Rat anti-mouse γc mAbs (4G3/3E12, IgG2b) were used as previously reported (Li *et al. J. Immunol.* 164:1193-1199, 2000).

Lymphocytes were labeled with fluorochrome 5-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Inc., Portland, OR) as follows. Spleens and peripheral lymph nodes were harvested from donor mice and single cell suspensions were prepared in Hanks balanced salt solution (HBSS). Red blood cells were lysed by hypotonic shock. Cells were resuspended in HBSS at $1x10^7$/ml and labeled with CFSE as described by Wells *et al.* (*J. Clin. Invest.* 100:3173-3183, 1997).

To activate CFSE-labeled T cells *in vivo,* DBA/2 mice were irradiated (1000 rad) with a Gammacell Exactor (Kanata, Ontario, Canada). Each mouse then received 4 to $6x10^7$ CFSE-labeled cells in 0.5 ml HBSS via the tail vein. Three days later, the host mice were sacrificed and spleens and peripheral lymph nodes were harvested separately. Single cell suspensions were prepared for cell surface staining and FACS analysis.

In some experiments, irradiated host mice were treated with anti-CD25 mAb or anti-γc mAbs (i.p. at 1mg/day for 3 days starting at i.v. injection of CFSE-labeled cells). Cell division *in vivo* was determined on the third day following injection of CFSE-labeled cells. Treatment with IL-15 mutant /Fc fusion protein consisted of 1.5 μg i.p. daily, for three days, starting at i.v. injection of labeled cells.

CFSE-labeled cells activated *in vivo* in irradiated allogeneic hosts were stained for the expression of IL-2 and IL-15 receptor subunits. To detect IL-2 receptor α chain expression, cells ($2x10^6$) were stained with PE-anti-mouse CD25 mAb on ice for 30 minutes, washed, and resuspended in 1 ml PBS containing 0.5% BSA. To detect IL-2R β and γc expression, cells were incubated with a rat anti-mouse β chain

(IgG2b) or γc mAb (IgG2b) on ice for 30 minutes, followed by incubation with a biotinylated anti-rat IgG2b. Cells were washed and further stained with PE-streptoavidin for 20 minutes. Cells were washed and resuspended in PBS-0.5% BSA for analysis. To detect IL-15R α chain expression, cells were incubated with an IL-15-FLAG fusion protein that binds to the α chain (Chae *et al.*, *J. Immunol.* 157:2813-2819, 1996) and then stained with biotinylated mouse anti-FLAG mAb. The cells were then washed and stained with PE-streptoavidin. Isotype matched control mAbs were included in each experiment as a control. All samples were analyzed using FACSort with CellQuest™ software (Becton Dickinson, Mountain View, CA). Data were collected and analyzed by gating onto $CFSE^+$ cells. All dividing $CFSE^+$ cells were T cells, as defined by the expression of CD3. At least 100,000 events were collected for each sample.

Apoptosis of dividing T cells *in vivo* was analyzed as follows. CFSE-labeled lymphocytes were stimulated *in vivo* in irradiated allogeneic hosts as described above. Cells were harvested from the host spleen or peripheral lymph nodes three days later and stained with PE conjugated annexin V on ice for 15 minutes in labeling buffer. Cell division was identified based on the cells' CFSE profile, and apoptotic cell death in each distinct cell division was analyzed by annexin V staining.

Cells were also stained for intracellular IL-2 and Bcl-2 cytokine expression. CFSE-labeled cells that had been activated *in vivo* for three days were harvested from the host spleen and lymph nodes. Cells were restimulated *in vitro* with PMA (50 ng/ml) and ionomycin (500 ng/ml) for four hours and GolgiStop™ (PharMingen) was added for the last two hours of culture. Cells were fixed and permeablized with Cytofix/Cytoperm (PharMingen) at 4°C for 10 minutes, and then stained with PE-conjugated anti-mouse IL-2 mAb, isotype matched control mAb was included as a control. For Bcl-2 staining, cells were fixed and permeablized with Cytofix/Cytoperm for 10 minutes and stained with PE-conjugated anti-Bcl-2 mAb or isotype control Ab for 30 minutes. Cells were washed and analyzed by FACS.

Cell sorting and *in vitro* re-stimulation was carried out as follows. CFSE-labeled cells were prepared from irradiated allogeneic hosts three days after i.v. injection of labeled cells. Cell proliferation *in vivo* was identified through analysis of their CFSE profiles. The second cell divisions were selected, gated, and sorted with

FACS Vantage™ sorter (Becton Dickinson) at 2000 events/second. The sorted cells were resuspended in RPMI 1640 medium supplemented with 10% FCS and 1% penicillin and streptomycin at $5x10^5$/ml and plated on anti-CD3 (2 μg/ml) coated plates along with anti-CD28 mAb (1 μg/ml). Three days later, cells were harvested and stained with PE-conjugated anti-mouse CD25 and isotype control Ab. Cell proliferation and IL-2 receptor α chain expression were analyzed by FACS.

Cell sorting and *in vitro* proliferation assays were carried out as follows. CFSE-labeled cells were prepared from irradiated allogeneic hosts three days after intravenous injection of labeled cells, and cell proliferation *in vivo* was identified by analysis of the cells' CFSE profile. The second cell division was selected, gated, and sorted with FACS Vantage™. Cells ($1x10^4$/ml) were resuspended in RPMI 1640 medium with 10% FCS and 1% penicillin and streptomycin, and stimulated with IL-2 (40 μ/ml to 500 μ/ml) or IL-15 (5 ng/ml) for 48 hours. Cells were pulsed with 1 mCi 3H-TdR (Amersham, Boston, MA) for 16 hours and ^{3}H-TdR uptake was determined by scintillation counting (Beckman Instrument, Columbia, MD).

The reagents and techniques described above provided the basis for several findings. First, CFSE-labeled B6AF1 (H-2b/d.k) allogeneic lymphocytes, in contrast to syngeneic controls (Li *et al.*, *Nature Medicine* 5:1298-1302, 1999), proliferated vigorously in irradiated DBA/2 (H-2d) hosts. Approximately 20% of the CFSE-labeled T cells recovered from the host spleen entered the cell cycle within three days of adoptive transfer, and seven to eight discrete rounds of cell division were clearly identified. Surprisingly, the IL-2 receptor α chain, which is required for high affinity IL-2 receptor signaling, could not be detected during the first 5 divisions, *i.e.*, this receptor subunit is expressed only after five cell divisions. In contrast, β subunits of the IL-2 receptor were expressed constitutively by all dividing T cells, and their level of expression was increased progressively as cells continued to divide. The pattern of γc expression *in vivo* differed strikingly from that of the α chain and the β chain. Undivided T cells (0 division) expressed very low levels of γ chain (<10%). Following entry into the cell cycle, γ chain was highly expressed by dividing T cells, and the levels of expression continued to increase after each consecutive cell division. After five cell divisions, however, γ chain expression was drastically down regulated, nearly reaching the basal level after the sixth cell division.

The differential expression of IL-2 receptor subunits is not due to selective accumulation of a subset of activated T cells in the host spleen, as CFSE-labeled cells harvested from peripheral lymph nodes displayed a remarkably similar pattern of expression for the three subunits of the IL-2 receptor.

Second, stimulation of CFSE-labeled T cells *in vitro* resulted in a uniform expression of all three subunits of the IL-2 receptor. This suggests that regulation of IL-2 receptor expression *in vivo* is distinct from that *in vitro*. The IL-2 receptor α chain is known to be sensitive to proteolytic cleavage *in vivo* in a manner that is similar to the selectins (Hemar *et al.*, *J. Cell. Biol.* 129:55-64, 1995). Staining for L-selectin expression by dividing T cells *in vivo* showed that L-selectin was expressed at high levels during the first five cell divisions, suggesting that the failure to detect IL-2 receptor α chain expression during the first five cell divisions is not due to rapid proteolytic cleavage. To determine whether T cells in the first five cell divisions are capable of expressing the IL-2 receptor α chain, T cells at the second cell division, which did not express IL-2 receptor α chain, were sorted and stimulated *in vitro* with immobilized anti-CD3 and soluble anti-CD28 for three days. These sorted T cells continued to divide upon *in vitro* restimulation, and all dividing T cells expressed the IL-2 receptor α chain. Clearly, expression of IL-2 receptor α chain *in vivo* and *in vitro* is differentially regulated.

As the receptor for IL-15 also uses the IL-2 receptor β and γ chains as critical signaling components (Tagaya *et al.*, *Immunity* 4:329-336, 1996), which are highly expressed during the first five cell divisions, we asked whether cells express an IL-15 receptor α chain that renders them responsive to IL-15 during initial cell divisions. Application of an IL-15-FLAG fusion protein as a primary staining reagent (Chae *et al.*, *J. Immunol.* 157:2813-2819, 1996), demonstrated that the IL-15 receptor α chain is clearly detectable, albeit at low levels, on dividing T cells regardless the number of cell divisions. The α chain for IL-2 receptor was not detected on all dividing T cells *in vivo*. Thus, selective expression of the α chain for IL-15 receptor, but not for IL-2 receptor, along with the expression of shared β and γ chains during the first five cell divisions, suggests that initial cell division *in vivo* is likely IL-15- but not IL-2-dependent.

To test this hypothesis, IL-2 production was assessed in dividing T cells *in vivo*. Intracellular IL-2 staining revealed that IL-2 was highly expressed only by cells that have divided more than five times. Treatment of host mice with saturating doses of cytolytic anti-CD25 mAb failed to inhibit the first five cell divisions (relative to control Ab treated mice), and dividing cells in the first and fifth divisions were remarkably similar in anti-CD25 treated mice and in control mice. Furthermore, T cells at the second cell division *in vivo* were sorted and cultured *in vitro* in the presence of IL-2 or IL-15, and cell proliferation was analyzed by ^{3}H-TdR uptake. IL-2, provided in doses as high as 500 u/ml in culture, failed to support T cell proliferation. In contrast, IL-15 stimulated vigorous cell proliferation.

The pattern of IL-2 expression *in vivo* is closely associated with upregulation of the IL-2 receptor α and β chains, and with markedly decreased expression of the common γ chain. This suggests that IL-2 regulates γ chain expression *in vivo*. To test this possibility, γ chain expression was examined in T cells from IL-2 deficient mice and wild type control mice. $CD4^{+}$ T cells from IL-2 deficient mice expressed very high levels of γ chain on the cell surface as compared to wild type controls. Treatment of host mice with anti-CD25 inhibited γ chain down regulation on dividing T cells *in vivo*, but this treatment had no effect on IL-2 receptor β chain expression.

The γ chain is a critical signaling element for all known T cell growth factors and γ chain signals are essential for cell survival, which is accomplished at least in part via sustained expression of Bcl-2 family anti-apoptotic proteins (Nakajima *et al.*, *J. Exp. Med.* 185:189-195, 1997). To determine whether decreased γ chain expression after five cell divisions *in vivo* regulates clonal expansion, CFSE-labeled cells were stained with PE-annexin V after recovery from the hosts and apoptotic cell death of dividing T cells was analyzed *in vivo*. Precipitous cell death occurred after four cell divisions. Undivided cells (0 division) had <10% annexin V positive cells. After the sixth cell division, however, ~40% of the cells were annexin V positive. This type of cell death is not Fas dependent, as T cells from Fas mutant MRL-lpr mice had similar pattern of apoptotic cell death *in vivo* (Li *et al.*, *J. Immunol.* 163:2500-2507, 1999). Staining for Bcl-2 expression showed that the mean channel fluorescence intensity of Bcl-2 staining was markedly decreased after four cell divisions. Thus, the signaling events upon γ chain down-regulation may fail to support sustained Bcl-2 expression

and cells become susceptible to apoptotic cell death (Nakajima *et al.*, *J. Exp. Med.* 185:189-195, 1997).

These results suggest that blocking IL-2 or IL-15 signaling will have different effects on T cell expansion *in vivo*. To explore this possibility further, CFSE-labeled lymphocytes from IL-2 deficient mice (H-2b) were injected into irradiated DBA/2 hosts (H-2d), cell division was analyzed *in vivo* three days later and compared with that in wild type control mice. T cells from IL-2 deficient mice continued to divide and expand *in vivo*. About 30% of CFSE-labeled cells entered the cell cycle, and the majority of the cells divided more than five times, compared to control.

Treating host mice with an IL-15 mutant /Fc, which acts as an IL-15 receptor specific antagonist (Kim *et al.*, *J. Immunol.* 161:5742-5748, 1998), markedly reduced the proliferation frequency of CFSE-labeled T cells, and an overwhelming majority of CFSE- labeled cells failed to enter the cell cycle in the treated mice. Furthermore, treatment of host mice with blocking mAbs against the common γ chain, a shared signaling component of IL-2 and IL-15 receptors, also markedly inhibited T cell division *in vivo*. Thus, IL-2 and IL-15 regulate distinct aspects of T cell expansion *in vivo*, and administration of antagonists for these interleukins can suppress the immune response, as discussed above.

These results also demonstrate that γ chain downregulation requires T cell activation and cell cycle progression as well as IL-2 signaling. Clearly, γ chain downregulation *in vivo* is closely associated with IL-2 production and high affinity IL-2 receptor expression. In the absence of IL-2, γ chain is expressed at extremely high levels and blockade of IL-2 receptor inhibits γ chain downregulation *in vivo* on cycling T cells. Thus, these studies provide novel evidence that IL-2 and IL-15 regulate distinct aspects of primary T cell activation *in vivo*. Contrary to traditional beliefs and conclusions based on *in vitro* studies, IL-15 is a critical growth factor in initiating T cell division *in vivo* and IL-2's unique role *in vivo* is to control the magnitude of clonal expansion by regulating γ chain expression on cycling T cells.

These results support the clinical applications described above. Attempts to boost T cell response with exogenous IL-2 in tumor immunity and AIDS may promote premature T cell death and therapies to block IL-2 in tolerance induction and autoimmunity may induce unwanted T cell expansion. Furthermore, staged and

combined targeting of IL-15 and IL-2 represent an important way to block T cell activation in T cell dependent cytopathic conditions.

Lytic IL-2/Fc lyses IL-2R-bearing cells and binds to FcRI

Cells of a T cell line (CTLL-2 cells; 10^6) were labeled with 100 mCi ^{51}Cr and incubated with a lytic form of IL-2/Fc and rat low-toxic complement (C'), a non-lytic form of IL-2/Fc and C', murine immunoglobulin and C'(a negative control) or C' alone (a negative control at 0.5 μg/ml). Another group of the same cells was treated with a detergent (1% NP40)(a positive control). Cell lysis was measured by ^{51}Cr release. The degree of lysis observed in the presence of the detergent represents 100% lysis. Specific lysis following treatment as described above was calculated according to the formula: % specific lysis = [(experimental cpm – background cpm)/(total release cpm – background cpm) x 100%]. The results support the conclusion that cytolytic IL-2/Fc lyses IL-2R-bearing CTLL-2 cells, but non-lytic IL-2/Fc does not.

To assess the ability of lytic and nonlytic IL-2/Fc to bind Fc receptors on FcRI-transfected CHO cells (murine FcRI, FcRII, and IL-2R-negative), FcRI transfectants were pre-incubated with PBS, mIgG2a, lytic IL-2/Fc, or nonlytic IL-2/Fc. After washing, fluorescent-conjugated goat anti-mouse Fc was used to stain the cells for FACS analysis. Cytolytic IL-2/Fc can bind FcRI, but lytic IL-2/Fc cannot.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A substantially pure polypeptide comprising a mutant interleukin-21 (IL-21) polypeptide comprising a mutation at position 114 or 119 of SEQ ID NO:2, wherein the mutant IL-21 polypeptide inhibits at least one of the cellular events that normally occurs when wild-type IL-21 specifically binds to an IL-21 receptor complex.

2. The polypeptide of claim 1, wherein the mutant IL-21 polypeptide inhibits at least one of the cellular events that normally occurs when a T cell receptor is activated.

3. The polypeptide of claim 1, wherein the mutation comprises a substitution mutation.

4. The polypeptide of claim 3, wherein the substitution mutation comprises deletion of a glutamine residue at position 114 or 119 and an addition, at the position(s) from which the glutamine residue was deleted, of one or more amino acid residues other than glutamine.

5. The polypeptide of claim 4, wherein the mutation comprises deletion of a glutamine residue at position 114 or 119 and an addition, at the position(s) from which the glutamine residue was deleted, of an aspartate residue.

6. The polypeptide of claim 1, wherein the mutant IL-21 polypeptide comprises a mutation at position 119 but not at position 114.

7. The polypeptide of claim 6, wherein the mutation at position 119 comprises substitution of an aspartate residue for a glutamine residue.

8. The polypeptide of claim 1, comprising a mutation at position 114 and a mutation at position 119.

9. The polypeptide of claim 8, wherein the mutation at position 114 comprises substitution of an aspartate residue for a glutamine residue.

10. The polypeptide of claim 8, wherein the mutation at position 119 comprises substitution of an aspartate residue for a glutamine residue.

11. The polypeptide of claim 1, further comprising a sequence that increases the circulating half-life of the mutant IL-21 polypeptide.

12. The polypeptide of claim 11, wherein the sequence that increases the circulating half-life of the mutant IL-21 polypeptide is albumin or a portion of an immunoglobulin molecule.

13. The polypeptide of claim 12, wherein the portion of an immunoglobulin molecule is an Fc region of an IgG molecule.

14. The polypeptide of claim 13, wherein the Fc region of the IgG molecule is lytic.

15. The polypeptide of claim 13, wherein the Fc region of the IgG molecule is non-lytic.

16. The polypeptide of claim 1, further comprising an antigenic tag.

17. The polypeptide of claim 16, wherein the antigenic tag is a FLAG sequence comprising Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQ ID NO:18).

18. An isolated nucleic acid molecule comprising a sequence that encodes the polypeptide of any of claims 1-17.

19. An expression vector comprising the nucleic acid molecule of claim 18.

20. A host cell comprising the expression vector of claim 19.

21. A physiologically acceptable composition comprising the polypeptide of any of claims 1-17, the nucleic acid molecule of claim 18, the expression vector of claim 19, or the host cell of claim 20.

22. A method of suppressing the immune response in a patient, the method comprising administering to the patient a therapeutically effective amount of a composition comprising an IL-21 antagonist or a nucleic acid that encodes an IL-21 antagonist.

23. The method of claim 22, wherein the IL-21 antagonist comprises the polypeptide of any of claims 1-17.

24. The method of claim 22, wherein the IL-21 antagonist comprises an antibody that specifically binds the IL-21 receptor complex.

25. The method of claim 22, wherein the patient has an autoimmune disease or is at risk of developing an autoimmune disease.

26. The method of claim 25, wherein the autoimmune disease is a rheumatic disease.

27. The method of claim 26, wherein the rheumatic disease is systemic lupus erythematosis, Sjögren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome, or Behcet's disease.

28. The method of claim 26, wherein the rheumatic disease is rheumatoid arthritis.

29. The method of claim 25, wherein the autoimmune disease is type I diabetes.

30. The method of claim 25, wherein the autoimmune disease is an autoimmune disease of the thyroid.

31. The method of claim 30, wherein the disease of the thyroid is Hashimoto's thyroiditis or Graves' disease.

32. The method of claim 25, wherein the autoimmune disease is an autoimmune disease of the central nervous system.

33. The method of claim 32, wherein the autoimmune disease of the central nervous system is multiple sclerosis, myasthenia gravis, or encephalomyelitis.

34. The method of claim 25, wherein the autoimmune disease is phemphigus vulgaris, phemphigus vegetans, phemphigus foliaceus, Senear-Usher syndrome, or Brazilian phemphigus.

35. The method of claim 25, wherein the autoimmune disease is psoriasis.

36. The method of claim 25, wherein the autoimmune disease is inflammatory bowel disease.

37. The method of claim 22, wherein the patient has received, or is scheduled to receive, a transplant of a biological organ, tissue, or cell.

38. The method of claim 22, wherein the patient has suffered a vascular injury.

39. A method of reducing the viability of an IL-21 receptor-bearing cell, the method comprising exposing the cell to a polypeptide of any of claims 1-17 under conditions in which the polypeptide can bind to the IL-21 receptor-bearing cell.

40. The method of claim 39, wherein the cell is malignant.

41. The method of claim 39, wherein the cell is a T cell, a B cell, or an NK cell.

42. A method of treating a patient who has been infected with a human immunodeficiency virus, the method comprising administering to the patient a therapeutically effective amount of a composition comprising an IL-21 antagonist or a nucleic acid that encodes an IL-21 antagonist.

43. A method of determining whether a patient has a disease or condition amenable to treatment with a polypeptide of any of claims 1-17, the method comprising

providing a biological sample from the patient;

exposing the sample to the polypeptide of claim 1, wherein the polypeptide further comprises an antigenic tag; and

determining whether the polypeptide specifically binds to the sample, the occurrence of binding indicating that cells within the sample can be bound by a mutant IL-21 polypeptide *in vivo* and that the patient has a disease or condition amenable to treatment with the polypeptide of claim 1.

44. A pharmaceutically acceptable composition comprising a first agent that specifically antagonizes an IL-21 receptor and a second agent that specifically binds a receptor for an interleukin other than IL-21.

45. The composition of claim 44, wherein the first agent is a polypeptide of any of claims 1-17

46. The composition of claim 45, wherein the polypeptide comprises an Fc region of an immunoglobulin.

47. The composition of claim 46, wherein the second agent comprises a mutant IL-15 polypeptide and an Fc region of an immunoglobulin molecule.

48. The composition of claim 46, wherein the second agent comprises an IL-2 polypeptide or a biologically active fragment or mutant thereof and an Fc region of an immunoglobulin molecule.

49. The composition of claim 45, wherein the second agent is an antibody that specifically binds, and thereby inhibits an activity of, an IL-15 receptor.

50. The composition of claim 45, wherein the second agent is (a) an antibody that specifically binds, and thereby acts as an agonist of, an IL-2 receptor or (b) an antibody that specifically binds, and thereby acts as an antagonist of, an IL-2 receptor.

51. The composition of claim 44, wherein the first agent is an antibody that specifically binds, and thereby inhibits an activity of, the IL-21 receptor.

52. The composition of claim 51, wherein the second agent is a mutant IL-15 polypeptide, optionally comprising an Fc region of an immunoglobulin molecule.

53. The composition of claim 51, wherein the second agent is IL-2 or a biologically active fragment or mutant thereof, optionally comprising an Fc region of an immunoglobulin molecule.

54. The composition of claim 51, wherein the second agent is an antibody that specifically binds, and thereby inhibits an activity of, an IL-15 receptor.

55. The composition of claim 51, wherein the second agent is an antibody that specifically binds, and thereby acts as an agonist of, an IL-2 receptor or an antibody that specifically bind, and thereby acts as an antagonist of, an IL-2 receptor.

56. The composition of claim 44, further comprising a third agent, wherein the third agent is an agent that inhibits cellular proliferation.

57. The composition of claim 56, wherein the agent that inhibits cellular proliferation is rapamycin.

58. The composition of claim 56, wherein the agent that inhibits cellular proliferation is mycophenolate mofetil.

59. A method of suppressing the immune response in a patient, the method comprising administering to the patient a therapeutically effective amount of the composition of claim 44 or of the composition of claim 56.

60. The method of claim 56, wherein the first agent is a polypeptide of any of claims 1-17.

61. The method of claim 60, wherein the polypeptide comprises an Fc region of an immunoglobulin.

62. The method of claim 61, wherein the second agent comprises a mutant IL-15 polypeptide and an Fc region of an immunoglobulin molecule.

63. The method of claim 61, wherein the second agent comprises an IL-2 polypeptide or a biologically active fragment or mutant thereof and an Fc region of an immunoglobulin molecule.

64. The method of claim 60, wherein the second agent is an antibody that specifically binds, and thereby inhibits an activity of, an IL-15 receptor.

65. The method of claim 60, wherein the second agent is an antibody that specifically binds, and thereby acts as an agonist of, an IL-2 receptor or an antibody that specifically binds, and thereby acts as an antagonist of, an IL-2 receptor.

66. The method of claim 59, wherein the first agent is an antibody that specifically binds, and thereby inhibits an activity of, the IL-21 receptor.

67. The method of claim 66, wherein the second agent is a mutant IL-15 polypeptide.

68. The method of claim 66, wherein the second agent is IL-2 or a biologically active fragment or mutant thereof.

69. The method of claim 66, wherein the second agent is an antibody that specifically binds, and thereby inhibits an activity of, an IL-15 receptor.

70. The method of claim 66, wherein the second agent is an antibody that specifically binds, and thereby acts as an agonist of, an IL-2 receptor or an antibody that specifically binds, and thereby acts as an antagonist of, an IL-2 receptor.

71. The method of claim 59, wherein the composition further comprises a third agent, wherein the third agent is an agent that inhibits cellular proliferation.

72. The method of claim 71, wherein the agent that inhibits cellular proliferation is rapamycin.

73. The method of claim 71, wherein the agent that inhibits cellular proliferation is mycophenolate mofetil.

74. A method of reducing the viability of an IL-21 receptor-bearing cell, the method comprising exposing the cell to an antibody against the IL-21 receptor under conditions in which the antibody can specifically bind to an IL-21 receptor on the IL-21 receptor-bearing cell.

75. A polypeptide comprising a mutant interleukin-21 (IL-21) polypeptide comprising a mutation at position 114 or 119 of SEQ ID NO:2

76. The polypeptide of claim 75, wherein the mutant IL-21 polypeptide binds to an IL-21 receptor complex.

77. The polypeptide of claim 75, wherein the mutant IL-21 polypeptide inhibits or suppresses an immune response.

78. The polypeptide of claim 75, wherein the mutant IL-21 polypeptide inhibits or suppresses the activation of T-cells.

```
hIL-21 --------MRSSPGNMERIVICLMVIFLGTLVHKSSSQGQDRHMIRMRQLIDIVDQLKNYVNDLV
mIL-21 ---------------MERTLVCLVVIFLGTVAHKSSPQGPDRLLIRLRHLIDIVEQLKIYENDLD
                                                  **** *:: * *::

hIL-15 MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKI-EDLI
hIL-4  ------------------MGLTSQLLPPLFFLLACAGNFVHGHKCD-ITLQEIIKTLNSLTEQKT
hIL-2  ----------------MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN

hIL-21 PEF-----------------LPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKR
mIL-21 PEL-----------------LSAPQDVKGHCEHAAFACFQKAKLKPSNPGNNKTFIIDLVAQLRR
                           :   ::   :  *:  ::       :    * *: *

hIL-15 QSMHIDAT------------LYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL
hIL-4  LCTELTVTDI----------FAASKNTTE----KETFCRAATVLRQFYSHHEKDTRCLGATAQQF
hIL-2  NYKN------PKLTRMLTFKFYMPKKATE---LKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDL

hIL-21 KPPSTNAGRRQKHRL------TCPSCDSYEKK--PPKEFLERFKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 1)
mIL-21 RLPARRGGKKQKHIA------KCPSCDSYEKR--TPKEFLERLKWLLQKMIHQHLS (SEQ ID NO: 2)
          :  *       *       :  :* *: :    ::::* : ***:  :
hIL-15 ANNSLSSNGNVTESG-------CKECEELEEK--NIKEFLQSFVHIVQMFINTS (SEQ ID NO: 3)
hIL-4  HRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERLKTIMREKYSKCSS (SEQ ID NO: 4)
hIL-2  ISNINVIVLGLKGSE------TTFMCE-YADETATIVEFLNRWITFCQSIISTL (SEQ ID NO: 5)
```

FIG. 1A

hIL-21.....ERFKSLLQ$_{114}$KMIHQ$_{119}$HLSSRTHGSEDS (SEQ ID NO: 6)

mIL-21.....ERLKWLLQ$_{114}$KMIHQ$_{119}$HLS (SEQ ID NO: 7)

hIL-15.....NIKEFLQ$_{101}$SFVHIVQ$_{108}$MFINTS (SEQ ID NO: 8)

hIL-2.....SFLNRWITFCQ$_{126}$SIISTL (SEQ ID NO: 9)

FIG. 1B

```
54/1                                                                84/11
atg gag agg acc ctt gtc tgt ctg gta gtc atc ttc ttg ggg aca gtg gcc cat aaa tca
M   E   R   T   L   V   C   L   V   V   I   F   L   G   T   V   A   H   K   S
114/21                                                              144/31
agc ccc caa ggg cca gat cgc ctc ctg att aga cct cgt cac ctt att gac att gct gaa
S   P   Q   G   P   D   R   L   L   I   R   L   R   H   L   I   D   I   V   E
174/41                                                              204/51
cag ctg aaa atc tat gaa aat gac ttg gat cct gaa ctt cta tca gct cca caa gat gta
Q   L   K   I   Y   E   N   D   L   D   P   E   L   L   S   A   P   Q   D   V
234/61                                                              264/71
aag ggg cac tgt gag cat gca gct ttt gcc tgt ttt cag aag gcc aaa ctc aag cca tca
K   G   H   C   E   H   A   A   F   A   C   F   Q   K   A   K   L   K   P   S
294/81                                                              324/91
aac cct gga aac aat aag aca ttc atc att gac ctc gtg gcc cag ctc agg agg agg ctg
N   P   G   N   N   K   T   F   I   I   D   L   V   A   Q   L   R   R   R   L
354/101                                                             384/111
cct gcc agg agg gga gga aag aaa cag aag cac ata gct aaa tgc cct tcc tgt gat tcg
P   A   R   R   G   G   K   K   Q   K   H   I   A   K   C   P   S   C   D   S
414/121                                                             444/131
tat gag aaa agg aca ccc aaa gaa ttc cta gaa aga cta aaa tgg ctc ctt caa aag atg
Y   E   K   R   T   P   K   E   F   L   E   R   L   K   W   L   L   Q   K   M
474/141                                                             504/151
att cat cag cat ctc tca gat ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa
I   H   Q   H   L   S   D   P   R   G   P   T   I   K   P   C   P   P   C   K
534/161                                                             564/171
tgc cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca aag atc aag
C   P   A   P   N   L   L   G   G   P   S   V   F   I   F   P   P   K   I   K
594/181                                                             624/191
gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt gtg gtg gtg gat gtg agc gag
D   V   L   M   I   S   L   S   P   I   V   T   C   V   V   V   D   V   S   E
654/201                                                             684/211
gat gac cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca gct cag
D   D   P   D   V   Q   I   S   W   F   V   N   N   V   E   V   H   T   A   Q
714/221                                                             744/231
aca caa acc cat aga gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc
T   Q   T   H   R   E   D   Y   N   S   T   L   R   V   V   S   A   L   P   I
774/241                                                             804/251
cag cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac aaa gac ctc
Q   H   Q   D   W   M   S   G   K   E   F   K   C   K   V   N   N   K   D   L
834/261                                                             864/271
cca gcg ccc atc gag aga acc atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta
P   A   P   I   E   R   T   I   S   K   P   K   G   S   V   R   A   P   Q   V
894/281                                                             924/291
tat gtc ttg cct cca cca gaa gaa gag atg act aag aaa cag gtc act ctg acc tgc atg
Y   V   L   P   P   P   E   E   E   M   T   K   K   Q   V   T   L   T   C   M
954/301                                                             984/311
gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag
V   T   D   F   M   P   E   D   I   Y   V   E   W   T   N   N   G   K   T   E
1014/321                                                            1044/331
cta aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc atg tac agc
L   N   Y   K   N   T   E   P   V   L   D   S   D   G   S   Y   F   M   Y   S
1074/341                                                            1104/351
aag ctg aga gtg gaa aag aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc
K   L   R   V   E   K   K   N   W   V   E   R   N   S   Y   S   C   S   V   V
1134/361                                                            1164/371
cac gag ggt ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt aaa tga
H   E   G   L   H   N   H   H   T   T   K   S   F   S   R   T   P   G   K   *
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(SEQ ID NO: 10)
(SEQ ID NO: 11)

FIG. 2

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54/1                                                    84/11
atg gag agg acc ctt gtc tgt ctg gta gtc atc ttc ttg ggg aca gtg gtc cat aaa tca
M   E   R   T   L   V   C   L   V   V   I   F   L   G   T   V   V   H   K   S
114/21                                                  144/31
agc ccc caa ggg cca gat cgc ctc ctg att aga ctt cgt cac ctt att gac att gtt gaa
S   P   Q   G   P   D   R   L   L   I   R   L   R   H   L   I   D   I   V   E
174/41                                                  204/51
cag ctg aaa atc tat gaa aat gac ttg gat cct gaa ctt cta tca gct cca caa gat gta
Q   L   K   I   Y   E   N   D   L   D   P   E   L   L   S   A   P   Q   D   V
234/61                                                  264/71
aag ggg cac tgt gag cat gca gct ttt gcc tgt ttt cag aag gcc aaa ctc aag cca tca
K   G   H   C   E   H   A   A   F   A   C   F   Q   K   A   K   L   K   P   S
294/81                                                  324/91
aac cct gga aac aat aag aca ttc atc att gac ctc gtg gcc cag ctc agg agg agg ctg
N   P   G   N   N   K   T   F   I   I   D   L   V   A   Q   L   R   R   R   L
354/101                                                 384/111
ccc gcc agg agg gga ggg aag aaa cag aag aaa cac ata gct aaa tgc cct tcc tgt gat tcg
P   A   R   R   G   G   K   K   Q   K   K   H   I   A   K   C   P   S   C   D   S
414/121                                                 444/131
tat gag aaa agg aca ccc aac gaa ttc cta gaa aga cta aaa tgg ctc ctt GAC aag atg
Y   E   K   R   T   P   K   S   F   L   E   R   L   K   W   L   L   D   K   M
474/141                                                 504/161
att cat GAC cat ctc tca gat ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa
I   H   D   H   L   S   D   P   R   G   P   T   I   K   P   C   P   P   C   K
534/161                                                 564/171
tgc cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca aag atc aag
C   P   A   P   N   L   L   G   G   P   S   V   F   I   F   P   P   K   I   K
594/181                                                 624/181
gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt gtg gtg gtg gat gtg agc gag
D   V   L   M   I   S   L   S   P   I   V   T   C   V   V   V   D   V   S   E
654/201                                                 684/211
gat gac cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca gct cag
D   D   P   D   V   Q   I   S   W   F   V   N   N   V   E   V   H   T   A   Q
714/221                                                 744/231
aca caa acc cat aga gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc
T   Q   T   H   R   E   D   Y   N   S   T   L   R   V   V   S   A   L   P   I
774/241                                                 804/251
cag cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac aaa gac ctc
Q   H   Q   D   W   M   S   G   K   E   F   K   C   K   V   N   N   K   D   L
834/261                                                 864/271
cca gcg ccc atc gag aga acc atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta
P   A   P   I   E   R   T   I   S   K   P   K   G   S   V   R   A   P   Q   V
894/281                                                 924/291
tat gtc ttg cct cca cca gaa gaa gag atg act aag aaa cag gtc act ctg acc tgc atg
Y   V   L   P   P   P   E   E   E   M   T   K   K   Q   V   T   L   T   C   M
954/301                                                 984/311
gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag
V   T   D   F   M   P   E   D   I   Y   V   E   W   T   N   N   G   K   T   E
1014/321                                                1044/331
cta aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc atg tac agc
L   N   Y   K   N   T   E   P   V   L   D   S   D   G   S   Y   F   M   Y   S
1074/341                                                1104/351
aag ctg aga gtg gaa aag aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc
K   L   R   V   E   K   K   N   W   V   E   R   N   S   Y   S   C   S   V   V
1134/361                                                1164/371
cac gag ggt ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt aaa tga
H   E   G   L   H   N   H   H   T   T   K   S   F   S   R   T   P   G   K   X
```

(SEQ ID NO: 12)
(SEQ ID NO: 13) FIG. 3

DNA sequence 489 b.p. atgagaatttcg ... aacacttcttga linear

```
1/1                                                    31/11
atg aga att tcg aaa cca cat ttg aga agt att tcc atc cag tgc tac ttg tgt tta ctt
Met arg ile ser lys pro his leu arg ser ile ser ile gln cys tyr leu cys leu leu
61/21                                                  91/31
cta aac agt cat ttt cta act gaa gct ggc att cat gtc ttc att ttg ggc tgt ttc agt
leu asn ser his phe leu thr glu ala gly ile his val phe ile leu gly cys phe ser
121/41                                                 151/51
gca ggg ctt cct aaa aca gaa gcc aac tgg gtg aat gta ata agt gat ttg aaa aaa att
ala gly leu pro lys thr glu ala asn trp val asn val ile ser asp leu lys lys ile
181/61                                                 211/71
gaa gat ctt att caa tct atg cat att gat gct act tta tat acg gaa agt gat gtt cac
glu asp leu ile gln ser met his ile asp ala thr leu tyr thr glu ser asp val his
241/81                                                 271/91
ccc agt tgc aaa gta aca gca atg aag tgc ttt ctc ttg gag tta caa gtt att tca ctt
pro ser cys lys val thr ala met lys cys phe leu leu glu leu gln val ile ser leu
301/101                                                331/111
gag tcc gga gat gca agt att cat gat aca gta gaa aat ctg atc atc cta gca aac aac
glu ser gly asp ala ser ile his asp thr val glu asn leu ile ile leu ala asn asn
361/121                                                391/131
agt ttg tct tct aat ggg aat gta aca gaa tct gga tgc aaa gaa tgt gag gaa ctg gag
ser leu ser ser asn gly asn val thr glu ser gly cys lys glu cys glu glu leu glu
421/141                                                451/151
gaa aaa aat att aaa gaa ttt ttg cag agt ttt gta cat att gtc caa atg ttc atc aac
glu lys asn ile lys glu phe leu gln ser phe val his ile val gln met phe ile asn
481/161
act tct tga
thr ser OPA
```

FIG. 4

DNA sequence 489 b.p. atgagaatttcg ... aacacttcttga linear

```
1/1                                             31/11
atg aga att tcg aaa cca cat ttg aga agt att tcc atc cag tgc tac ttg tgt tta ctt
Met arg ile ser lys pro his leu arg ser ile ser ile gln cys tyr leu cys leu leu
61/21                                           91/31
cta aac agt cat ttt cta act gaa gct ggc att cat gtc ttc att ttg ggc tgt ttc agt
leu asn ser his phe leu thr glu ala gly ile his val phe ile leu gly cys phe ser
121/41                                          151/51
gca ggg ctt cct aaa aca gaa gcc aac tgg gtg aat gta ata agt gat ttg aaa aaa att
ala gly leu pro lys thr glu ala asn trp val asn val ile ser asp leu lys lys ile
181/61                                          211/71
gaa gat ctt att caa tct atg cat att gat gct act tta tat acg gaa agt gat gtt cac
glu asp leu ile gln ser met his ile asp ala thr leu tyr thr glu ser asp val his
241/81                                          271/91
ccc agt tgc aaa gta aca gca atg aag tgc ttt ctc ttg gag tta caa gtt att tca ctt
pro ser cys lys val thr ala met lys cys phe leu leu glu leu gln val ile ser leu
301/101                                         331/111
gag tcc gga gat gca agt att cat gat aca gta gaa aat ctg atc atc cta gca aac aac
glu ser gly asp ala ser ile his asp thr val glu asn leu ile ile leu ala asn asn
361/121                                         391/131
agt ttg tct tct aat ggg aat gta aca gaa tct gga tgc aaa gaa tgt gag gaa ctg gag
ser leu ser ser asn gly asn val thr glu ser gly cys lys glu cys glu glu leu glu
421/141                                         451/151
gaa aaa aat att aaa gaa ttt ttg gac agt ttt gta cat att gtc gac atg ttc atc aac
glu lys asn ile lys glu phe leu asp ser phe val his ile val asp met phe ile asn
481/161
act tct tga
thr ser OPA
```

FIG. 5

CREATION OF A CYTOLYTIC IL-21/Fc FUSION PROTEIN

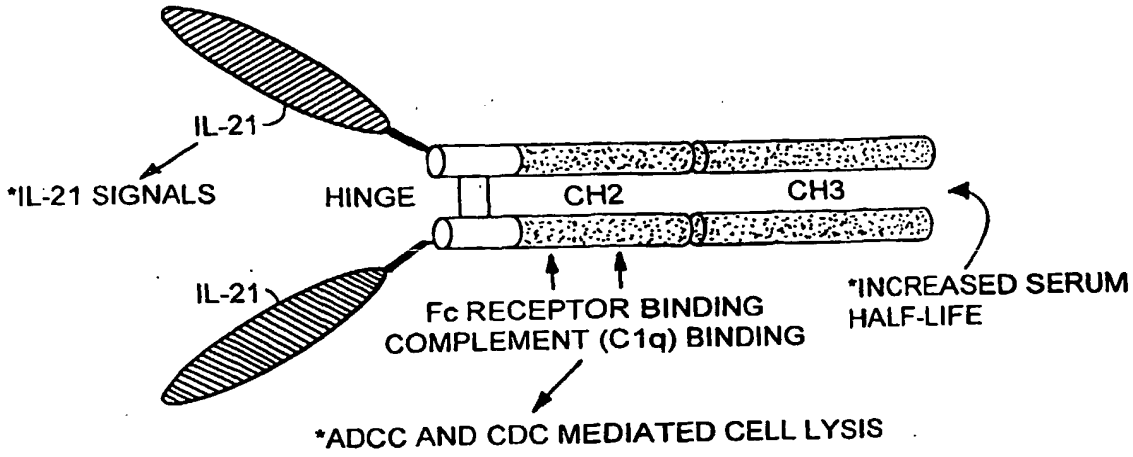

FIG. 6A

CREATION OF A CYTOLYTIC MUTANT IL-21/Fc FUSION PROTEIN

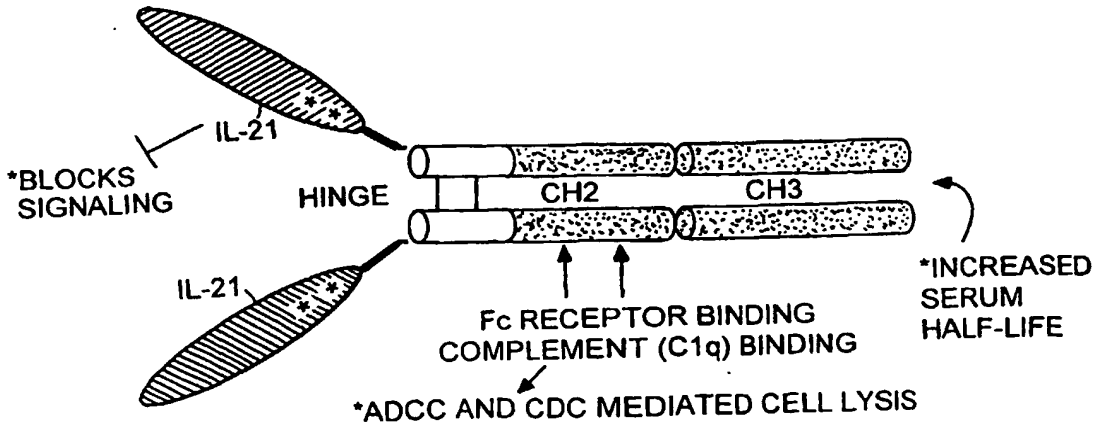

FIG. 6B

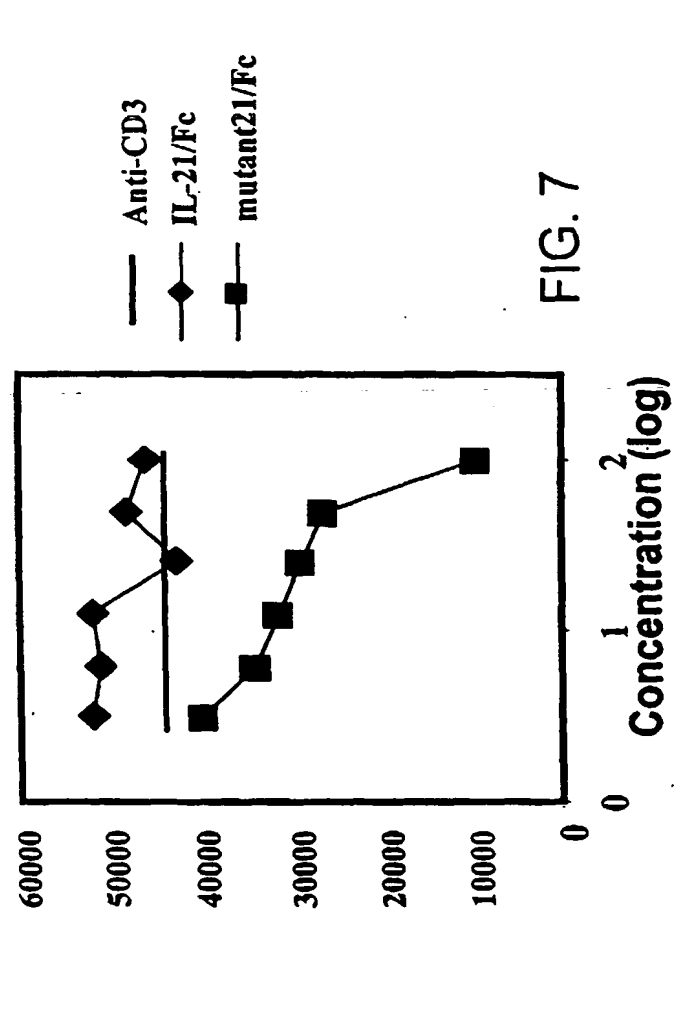
Antagonist Mutant IL-21/Fc, but not IL-21/Fc,
Blocks Anti-CD3 triggered PBMC Proliferation in vitro
Anti-CD3
IL-21/Fc
mutant21/Fc
60000
50000
40000
30000
20000
10000
0
0
1
2
Concentration (log)

FIG. 7

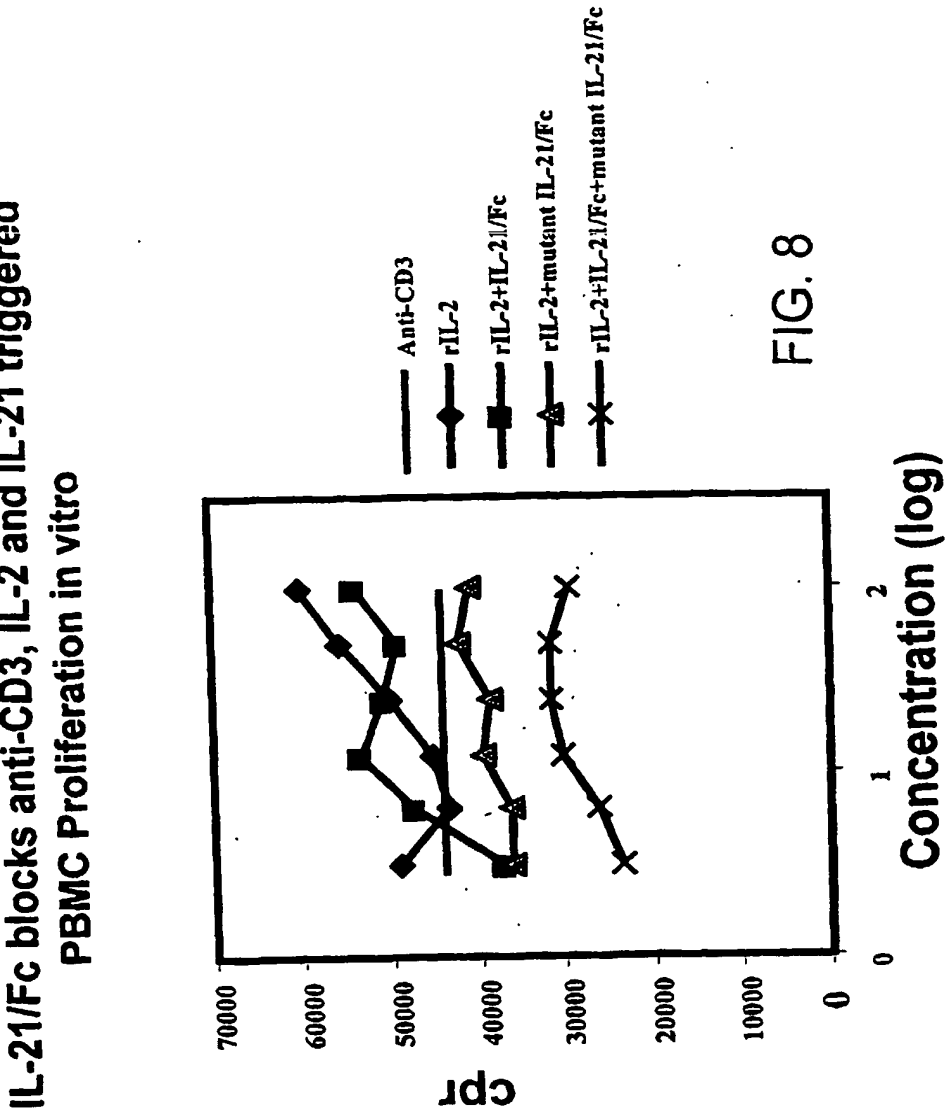
Mutant IL-21/Fc blocks anti-CD3, IL-2 and IL-21 triggered
PBMC Proliferation in vitro
cpr
70000
60000
50000
40000
30000
20000
10000
0
0
1
2
Concentration (log)
Anti-CD3
rIL-2
rIL-2+IL-21/Fc
rIL-2+mutant IL-21/Fc
rIL-2+IL-21/Fc+mutant IL-21/Fc

FIG. 8

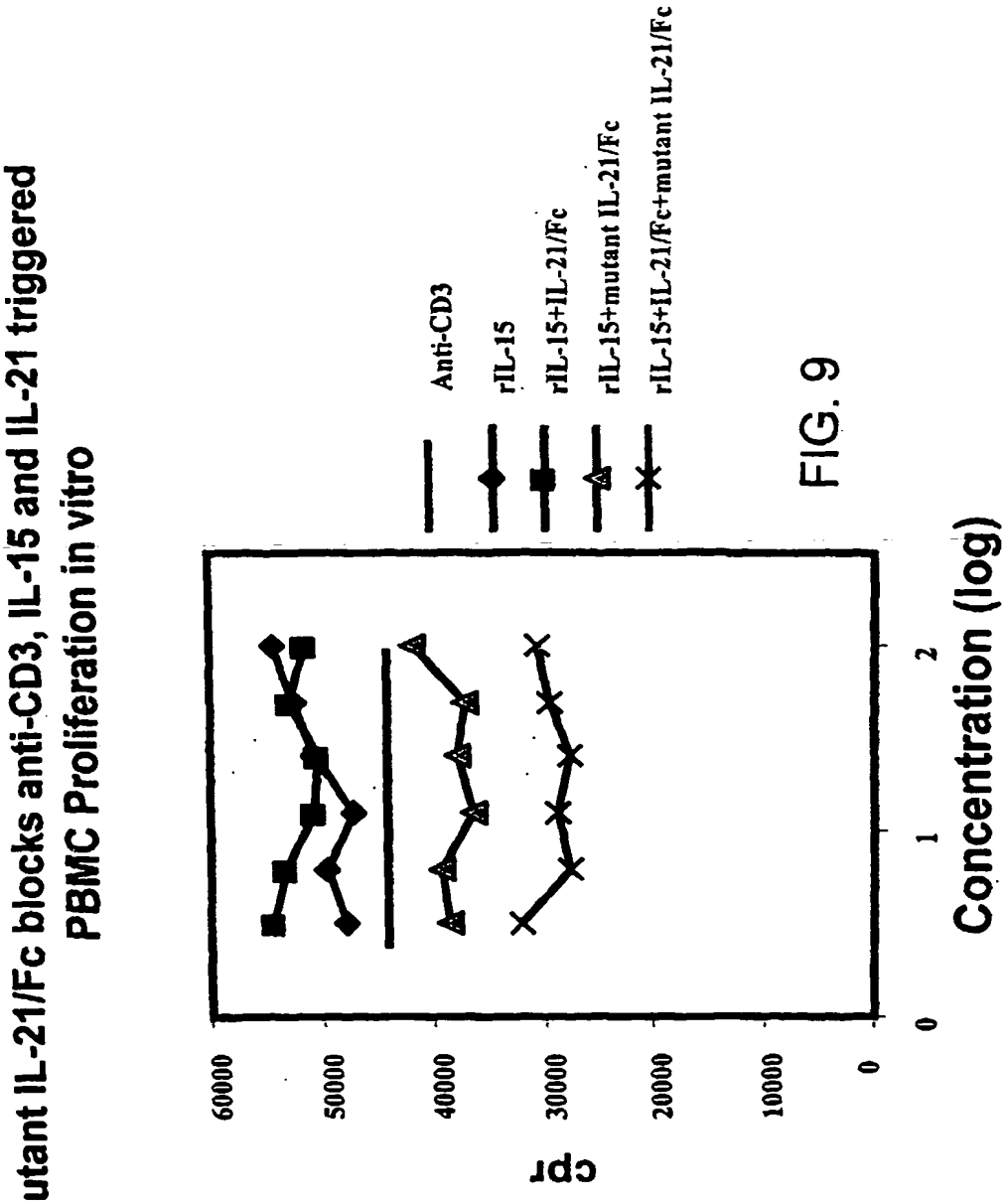
Mutant IL-21/Fc blocks anti-CD3, IL-15 and IL-21 triggered PBMC Proliferation in vitro
Anti-CD3
rIL-15
rIL-15+IL-21/Fc
rIL-15+mutant IL-21/Fc
rIL-15+IL-21/Fc+mutant IL-21/Fc
cpr
60000
50000
40000
30000
20000
10000
0
0
1
2
Concentration (log)

FIG. 9